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ON THE OCCURRENCE AND PHYSIOLOGICAL SIGNIFICANCE OF FAT IN THE MUSCLE FIBERS OF THE NORMAL MYOCARDIUM AND ATRIO-VENTRICULAR SYSTEM: INTERSTITIAL GRANULES (MITOCHONDRIA) AND PHOSPHOLIPINES IN CARDIAC MUSCLE

H. HAYS BULLARD

From the Anatomical Laboratories, School of Medicine, University of Pittsburgh

SIXTEEN FIGURES¹

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INTRODUCTION

In this paper I shall consider the occurrence and physiological significance of microscopically demonstrable neutral fat in the fibers of normal cardiac muscle and shall also discuss briefly the so-called true interstitial granules and their relation to the phospholipines of the heart. I have examined more than two hundred apparently normal mammalian hearts. Of these, the one hundred and forty-four last studied are listed below in tabular form.

Normal cardiac muscle fibers, like other tissues of the body, contain a very considerable percentage of fats. This important fact has been determined by Krehl ('93), Rosenfeld ('01), Leick

¹A large part of the cost of illustrations was borne by the Anatomical Laboratories, University of Pittsburgh.

and Winckler ('02), Rubow ('04-'05), Erlandsen ('07), Rosenbloom ('13 a) and others who have analyzed portions of the myocardium which had been carefully freed from adipose tissue. The work of the biochemists just mentioned indicates that from two to four per cent of the weight of the undried substance of the myocardium is fat, half of which is present as phospholipines while the other half is mainly neutral fat. The entire fat content of normal cardiac muscle is commonly supposed to be 'invisible,' that is, in such a form that it cannot be microscopically demonstrated. A few investigators would appear to make the total fat content not only 'invisible' but 'combined' or 'masked;' others would limit the latter terms to the comparatively small fraction of the total fats which can be extracted only after protein digestion. According to Leathes ('10) there is not sufficient evidence to justify us in assuming the existence of a chemical combination of fat and protein. From Rosenbloom's ('13 b) recent review of the literature of the subject one is impressed by the vague character of the information as yet vouchsafed concerning this supposed fat-protein compound.

Virchow ('47) taught that visible fat appears in cardiac muscle only as the result of a pronounced retrogressive process, the degeneration of cell protein into fat. By many clinicians, 'fatty degeneration' in the heart was thought to lead to serious disturbances of function of the organ with death as the inevitable outcome. Welch ('88) demonstrated that this extreme view is not tenable. He observed that rabbits kept for some time at a high temperature and therefore known to possess fatty cardiac muscle, show no symptoms of cardiac derangement either at the time of the experiment or thereafter. He also found that cardiac fibers which are crowded with fat will nevertheless contract rhythmically. Flexner ('94-'95) showed that fat in the heart muscle appears more frequently in certain infections than in others. He pointed out that many clinical conditions formerly supposed to be due to fatty degeneration of the heart are now known to be due to other causes. Hasenfeld and Fenyvessy ('99) demonstrated that even when cardiac muscle is extremely fatty, following phosphorus poisoning, the heart action is apparently unimpaired.

They interpreted this apparently normal action of the heart, in the presence of a marked fatty change, as due to the very large reserve force of the cardiac fibers. Pratt ('04) concluded that there is no evidence to support the once commonly accepted theory that fatty metamorphosis of the heart muscle is often the cause of myocardial insufficiency. Many other observers have arrived at similar conclusions and nearly all now agree with the view advanced by Rosenfeld ('01) and Herxheimer ('02) that the fat in so-called fatty degeneration or metamorphosis is in reality of infiltrative, not degenerative origin. Of importance in this connection are the observations of Lambert ('14) upon tissue cultures of the chick heart. He finds that the source of the fat in the embryonic muscle cells is the medium in which the cells grow and that the fat droplets are not the result of cell degeneration.

Referring especially to fat in the heart, Mallory ('14) has recently well expressed the view now held by most pathologists:

The fat makes its appearance in visible form because of diminished utilization (oxidation) of the fat normally brought to the muscle cytoplasm. The fat accumulates as the result of two different causes, (a) disturbances of nutrition and (b) toxemia. The accumulation of fat in the cytoplasm of the muscle-fibers has of itself little significance. It may in time unquestionably be utilized and removed. Its importance pathologically lies in the evidence its presence gives of disturbed cell metabolism and in its frequent association with necrosis.

Ostertag ('89), Ricker and Ellenbeck ('99), Fibiger ('01), Arnold ('03), Keimath ('04) and Babes ('08) have referred to the isolated occurrence of visible fat in the apparently normal cardiac fibers of certain species or individuals either without advocating or without offering sufficient proof that fatty droplets are of usual occurrence in this situation.

Hofbauer ('05) describes visible fat in normal human fetal heart muscle. Bell ('11) was the first to show clearly that visible fat ('liposomes') is normally present in the cardiac muscle of the common laboratory mammals. He also demonstrated that the quantity of visible fat is increased when fatty foods are given and decreased when animals are starved. Bell's work, as well as my own observations in confirmation of his results ('12 a), had ref-

erence mainly to skeletal muscle but we both stated that our results also applied to cardiac muscle. Wegelin ('13), working independently, obtained experimental results similar to those which Bell and I had previously published. Wegelin gives one drawing showing fat in the heart muscle of a normal rat. With this single exception there are, in the literature, no figures purporting to show the normal fat content of cardiac muscle.

In the recent literature are two important communications, by Eyselein ('14) and Borchers ('14). Both of these authors are familiar with the work of Wegelin but neither is able to confirm his results. In general, the observations recorded in the present paper are in agreement with those of Hofbauer ('05), Bell ('11-'12), Wegelin ('13), and with my own previous work ('12-'14).

I am indebted to Prof. R. E. Sheldon for kind encouragement and valuable criticism in connection with the work of this paper. In making the figures I have received a number of helpful suggestions from Miss S. E. Watson, artist of the Department of Anatomy.

METHODS

Technique of fat demonstration. In the demonstration of fats in tissue sections, methods and technique are of the utmost importance. The wide divergence of opinion among different investigators concerning the occurrence of fat within the cells of normal and of pathological tissues is due primarily to differences of technique in preparing the sections for examination. I have elsewhere ('12 b) treated this subject in some detail and shall here give only a brief outline.

Fixation. Fat containing material is usually fixed in formalin. Bell ('11) and Bullard ('12) have pointed out that this fixative, although frequently giving excellent results, is not to be relied upon under all circumstances. Frozen sections of the heart or other tissues which have been fixed in formalin may, when carefully stained (Scharlach R.), appear to contain little fat, while sections of the same material when stained fresh without previous fixation may be loaded with droplets. This apparent disappear-

ance of fat may be noticed when the blocks of tissue have remained in the fixative only a few hours, at other times it occurs only after several weeks or months, if at all.

As a fixative I now employ formalin which has been neutralized and distilled according to the method given by Mann ('02) in his *Physiological Histology*, p. 88. A twenty per cent solution of formalin is prepared and is rendered isotonic by the addition of 0.75 gm. of sodium chloride to each 100 cc. of the fluid. With short fixation in this solution the quantity of fat usually does not differ from that seen in fresh tissue. Blocks are fixed for thirty minutes to five hours and are then cut on the freezing microtome. Frozen sections of fresh unfixed tissue are employed as controls.

Staining. In this study I have employed all the fat stains in common use but principally Herxheimer's alkaline alcoholic solution of Scharlach R., which stain usually shows much more fat than the simple alcoholic solutions of the same dye. The latter solutions sometimes fail to stain a large part of the fatty droplets seen in the fresh unstained tissue. This is not the case with Herxheimer's solution. Herxheimer's stain is a saturated solution of Scharlach R. in seventy per cent alcohol to which sodium hydroxide, 2 gm. per 100 cc. has been added. It is essential that care be taken to avoid precipitates. Also the excess stain must be thoroughly washed out of the sections before the nuclei are stained with dilute hematoxylin. For the details of this method the reader is referred to Herxheimer's papers ('01-'02), or to my former papers ('12).

DIFFERENTIATION OF NEUTRAL FAT

Among recent contributions to our knowledge of the technique and chemistry of fat demonstration may be mentioned those of Herxheimer ('01), J. Lorraine Smith ('06-'07-'10), Smith, Mair and Thorpe ('08), Smith and Mair ('10), Fischler ('04), Fauré-Fremiet, Mayer and Schaeffer ('10), Eisenberg ('10), Klotz ('06), Aschoff ('09), Kawamura ('11) and Hanes and Rosenbloom ('11). These observers have introduced a number of valuable staining methods but more important still they have established a large

number of facts relating to the optical, chemical and physical properties exhibited by pure fats and fatty mixtures when observed after being artificially introduced into the tissues or when studied as smears on tissue paper. Application of the knowledge thus gained makes possible, in certain cases, the identification of various fats as they occur in the tissues.

Figures 1, 2, 3, 4, and 5 represent sections of the myocardium of rats, figure 10 is from a dog and figure 9 from a fattened hog. The preparations were stained with Herxheimer's Scharlach R. The number of normal hearts which I have examined by this method is more than two hundred and always with results similar to those represented in the figures. It is of course well recognized that Scharlach R. is not specific for neutral fat. Nevertheless, I believe that the colored droplets shown in these figures are, at least in very large part, neutral fat. The reasons for this belief as outlined below are essentially those advanced in a former paper ('12 a). Concerning similar fatty droplets in cardiac muscle Wegelin ('13), likewise, has come to the conclusion that they are neutral fat and for much the same reasons.

In unstained preparations these droplets are to be seen as approximately spherical, highly refractive, isotropic bodies which do not disappear in acetic acid or in dilute alkalies, form no myelin figures, but are completely dissolved by absolute alcohol and other fat solvents. They stain characteristically with Scharlach R. and stain red, not blue, with Nile blue sulphate and Nile blue chlorhydrate. They do not stain with basic anilin dyes and are not rendered insoluble by the action of potassium bichromate. The above combination of properties makes it appear certain that the droplets under consideration are neutral fat and not any of the other fats occurring either normally or pathologically in cardiac muscle as, phospholipines, cholesterol-ester, etc.

RELATION OF NEUTRAL FAT TO STRUCTURE OF CARDIAC MUSCLE

Position of fat in muscle fibers. Virchow ('47) pointed out that the pathological fat droplets of cardiac muscle fibers are situated in the sarcoplasm, not in the fibrillae or muscle columns. This view has been confirmed by nearly all subsequent observers. Welch ('88) observed that the droplets are arranged in rows between the fibrillae and according to Wegelin ('13) transverse rows are found in segment J on either side of the membrane of Krause.

In well stained preparations of normal cardiac muscle, made by the Herxheimer method, it is very clearly seen that the fat droplets are found in the sarcoplasm, never in the muscle columns or myo-fibrillae. The droplets are arranged in both longitudinal and transverse rows, figure 5. When the muscle fiber is contracted the larger droplets, about $2\ \mu$ in diameter, are spherical and each occupies an entire segment of the fiber extending between adjacent Krause's membranes. When the fiber is extended the large droplets are usually somewhat elongated and are found in the anisotropic segment Q. Small droplets, $1\ \mu$ or less in diameter, are arranged in transverse rows in the isotropic segment J on either side of Krause's membrane.

Light and dark fibers. As regards distribution of affected fibers, Ribbert ('97) recognized three types of fatty degeneration: 1) diffuse general degeneration in which all the fibers contain fat; 2) mottled peri-arterial degeneration affecting areas immediately surrounding the smaller arteries; 3) mottled degeneration occurring in areas most distant from the smaller arteries. This latter type gives the well known tiger-lily or thrush-breast appearance usually most marked in the papillary muscles.

In normal cardiac muscle the fatty fibers do not give rise to the mottled appearance but conform closely to the diffuse general type of Ribbert. All of the fibers of a specimen and of the whole heart may show a uniform amount of fat, figure 1, and figure 6 at C. In an equal number of individuals certain of the fibers contain much more fat than others, figures 3, 4, 5, 9, 10, and 13. This latter distribution of the fat droplets, at times observed in all the species (rat, cat, dog, hog, sheep, ox, man) here studied, is

similar to that occurring in the so-called light and dark fibers of skeletal muscle.

The existence of light, dark and intermediate fibers (by transmitted light) has been known in skeletal muscle for a long time. Knoll ('89-'91) and Schaffer ('93) described the dark fibers as containing many interstitial granules and fatty droplets while the light fibers contained comparatively few granules and little fat. Figure 14 shows a section of striated muscle fibers from the diaphragm of a dog. At D is seen a fatty or dark fiber, and at L a slightly fatty or light fiber. The work of Knoll and Schaffer was done before Scharlach R. was extensively used as a fat stain so that they did not obtain the exact picture shown in figure 14 but there can be little doubt that the types of voluntary striated fibers here shown (fig. 14) correspond to the light and dark fibers of Knoll. In skeletal muscle the fibers are of uniform type, either light or dark throughout their entire length, and as was pointed out by Knoll it is easy, in certain cases, to observe morphological differences between the two types. A good example of this is found in the breast muscle of the pigeon. Here the light fibers are large, with nuclei placed within their substance and contain little fat, while the dark fibers are small, with nuclei peripherally situated, and contain a great deal of fat. It is certain that in the pigeon light and dark fibers are definitely fixed types and not morphologically identical. In the skeletal muscles of mammals a morphological difference is often observed in that the dark fibers are of less diameter than the light. In a former paper ('12 a) I reported having observed the two types of fibers in the skeletal muscles of the human fetus and in the fetal calf. I have not been able to differentiate the two types during the first half of fetal life but in the human fetus they are well marked as early as the sixth and seventh month. The relative number and arrangement of the dark (fatty) fibers in the different muscles of the human fetus is so similar to that found in the adult that it seems certain that the dark fibers of the fetus remain true to type in post-uterine life. In the mammalian fetal heart usually the different types of fibers are not clearly marked although they contain fat droplets.

According to Knoll ('91) and Schaefer ('12) cardiac muscle fibers correspond to the dark fibers of skeletal muscle. It has been my experience, however, that in many cases the different types of fibers, dark, light and intermediate are quite as well marked in cardiac as in skeletal muscle. Figures 3, 4, and 5 show cardiac fibers of the rat, figure 10 those of a dog and figure 9 those of a fattened hog. The different types of fibers are clearly shown in each of these figures. Dark fibers are designated D, light fibers L. Figure 5 shows a longitudinal section from the same specimen as the transverse section shown in figure 4. In the longitudinal section it is seen that after a brief course fibers of one type, dark or fatty, pass abruptly into those of different type, light or slightly fatty. This change of type occurs along the transverse lines marked out by the intercalated disks. The greater number of intercalated disks, however, mark no change of type. A cardiac fiber of any given type includes, therefore, a variable number of so-called cardiac cells. In inanition, as we shall see, fat gradually disappears from heart muscle. All the fibers then appear light and it is frequently impossible to distinguish one type from the other, figure 1. Similarly when the muscle fibers, as in certain fat fed animals, are loaded with fat the light fibers may be so crowded with fat droplets as to present the same appearance as dark fibers, figure 6.

In the skeletal muscles of nearly all apparently normal mammals including man, dark or fatty fibers are found, almost invariably, side by side with others which are light or non-fatty and as I have set forth the two types are also of frequent occurrence in apparently normal cardiac muscle. This indicates that dark or fatty fibers are to be considered normal, not pathological. The occurrence of light and dark fibers is usually accounted for on the theory that dark fibers have undergone pathological 'fatty degeneration' while light fibers have escaped the pathological process. This explanation we cannot accept for reasons given, as well as for others to be stated later.

Distribution of fatty fibers in the heart. The hearts of the rats here used were usually prepared for study by making frozen sections extending transversely across both ventricles. Such

preparations bring out the fact that the fatty fibers are distributed with approximate uniformity throughout the myocardium of both ventricles. This is also true in the cat and doubtless in other animals although I have not studied the question exhaustively. In the auricles the fat content of the fibers appears normally to parallel that found in the ventricles. Figure 13 represents a transverse section of fibers from the right auricle of a dog. Figure 10 at L and D shows cardiac fibers from the interventricular septum of the same heart. The number of fat droplets and the distribution of light and dark fibers in the auricle is similar to that in the ventricle.

OCCURRENCE OF NEUTRAL FAT IN THE HEART MUSCLE OF DIFFERENT MAMMALS UNDER VARIOUS NUTRITIVE CONDITIONS

The data upon which this investigation rests are, in part, given below in tabular form. The animals are grouped according to species and also with respect to character of food. Although in nearly all animals some cardiac fibers hold much more fat than others, the distribution of fatty fibers is so uniform that in any given heart the quantity of fat in sections taken at random from different parts of the ventricles is approximately the same in all sections. This makes it possible, in any given individual, to represent fairly accurately the amount of fat in the ventricular fibers by one of the following five designations viz: *very large*, figure 6; *large*, figure 4; *moderate*, figure 3; *small*, figure 2; *very small*, figure 1. An acquaintance with the literature makes it appear that what is 'very large' to one author is but 'large' to another and 'moderate' to a third. In order to show with some clearness what is here intended to be conveyed by the various designations just given a type drawing is referred to in each case. From the tables it will at once be noted that animals, especially rats, kept for a short time on a fatty diet, have much more fat in the heart muscle than do those which are on a diet of carbohydrate and protein with only a small amount of fat. In inanition, however, animals show a comparatively small amount of fat in the cardiac fibers. The fat findings here given are based upon preparations stained by Herxheimer's alkalin-alcoholic

Scharlach R. The various items of the tables will be more fully explained in the discussion which follows.

Group 1 (table 1) consists of fifteen adult rats fed for three weeks with an abundance of raw grain, wheat bread and boiled beef. The quantity of fat allowed was somewhat less than the animals appeared to desire. This group of rats may be considered as having received food, fat, protein, and carbohydrate, suitable for normal maintenance and growth. These rats were kept in large well ventilated cages and when killed were all in good nutritive condition. Of the fifteen rats in the group (table 1) ten have a moderate amount of fat in the cardiac fibers as in figure 3; two have a large amount of fat in the fibers as in figure 4, three have a small amount as in figure 2. Figure 3, showing as it does a moderate amount of fat, may be taken as representing the average condition of the group.

TABLE 1

*Albino rats, normally fed group, kept
for three weeks on wheat bread, raw
grain, and boiled beef with a small
quantity of fat*

| ANIMAL NUMBER | WEIGHT IN GRAMS WHEN KILLED | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (SMALL AS IN FIG. 2) (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) |
|---------------|--------------------------------|--|
| | | |
| 1 | 126 | moderate |
| 2 | 178 | large |
| 3 | 137 | moderate |
| 4 | 185 | moderate |
| 5 | 176 | small |
| 6 | 196 | moderate, fig. 3 |
| 7 | 135 | moderate |
| 8 | 187 | large |
| 9 | 139 | moderate |
| 10 | 187 | small, fig. 2 |
| 11 | 196 | moderate |
| 12 | 135 | moderate |
| 13 | 143 | small |
| 14 | 156 | moderate |
| 15 | 163 | moderate |

Group 2 (table 2) consists of ten rats in various stages of inanition. As is seen from the table these rats had been without food for forty-eight to ninety-six hours and had lost from twelve to twenty-four per cent in body weight. The three members of the group which had lost as much as twenty per cent in weight showed, upon section, little or no subcutaneous fat and but slight traces in the omentum. Several of these animals are to be regarded as in the last stages of inanition. Of the ten members of the group five have a very small quantity of fat in the cardiac fibers, as in figure 1, four have a small amount, as in figure 2, and one a moderate amount. Figure 1 from rat no. 23 (loss of weight twenty per cent) shows a very small amount of fat and this figure may be taken as characteristic of the inanition group.

The animals of group 3 (table 3) were fed fats in the form of butter, olive oil, pork fat and egg yolk. In order to increase the quantity of fat consumed no food was given for twenty-four hours preceding the initial feeding. When fats were to be given for several days, feeding was not preceded by a fast. A little grain

TABLE 2

Albino rats, inanition group, rats 16-20 inclusive, no food for 48 hours; rats 21 and 22, no food for 60 hours; rats 23-25 inclusive, no food for 96 hours, water supplied

| ANIMAL NUMBER | WEIGHT IN GRAMS WHEN KILLED | LOSS OF WEIGHT IN PER CENT | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (MODERATE AS IN FIG. 3) (SMALL AS IN FIG. 2) (VERY SMALL AS IN FIG. 1) |
|---------------|-----------------------------|----------------------------|--|
| 16 | 147 | 12 | small |
| 17 | 173 | 14 | very small |
| 18 | 116 | 14 | small |
| 19 | 148 | 13 | small |
| 20 | 137 | 13 | moderate |
| 21 | 176 | 15 | small |
| 22 | 99 | 16 | very small |
| 23 | 185 | 20 | very small, fig. 1 |
| 24 | 96 | 24 | very small |
| 25 | 90 | 23 | very small |

was given in nearly all cases for the reason that it appears to stimulate the appetite of the animals for fats. It is known, moreover, that fat metabolism does not proceed normally in the absence of carbohydrates. As shown in table 3 the thirty-

TABLE 3

Albino rats, fat fed group. The rats of this group were given all the fatty food that they would eat plus a small amount of raw grain

| ANIMAL NUMBER | WEIGHT IN GRAMS WHEN KILLED | FATTY FOOD GIVEN | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) |
|------------------|-----------------------------------|------------------------|--|
| 26 | 156 | Butter for 10 hours | large |
| 27 | 109 | Butter for 10 hours | large |
| 28 | 188 | Butter for 20 hours | large |
| 29 | 167 | Butter for 20 hours | large |
| 30 | 179 | Butter for 20 hours | large |
| 31 | 112 | Butter for 36 hours | moderate |
| 32 | 146 | Butter for 48 hours | large |
| 33 | 179 | Butter for 14 days | large |
| 34 | 183 | Butter for 14 days | moderate |
| 35 | 186 | Butter for 14 days | large |
| 36 | 194 | Butter for 14 days | moderate |
| 37 | 173 | Butter for 7 days | large |
| 38 | 115 | Butter for 7 days | moderate |
| 39 | 147 | Olive oil for 20 hours | moderate |
| 40 | 179 | Olive oil for 20 hours | large |
| 41 | 168 | Olive oil for 20 hours | moderate |
| 42 | 114 | Olive oil for 20 hours | moderate |
| 43 | 164 | Pork fat for 10 hours | large |
| 44 | 183 | Pork fat for 15 hours | large |
| 45 | 198 | Pork fat for 20 hours | moderate |
| 46 | 162 | Pork fat for 20 hours | large, figs. 4 and 5 |
| 47 | 174 | Pork fat for 36 hours | large |
| 48 | 195 | Pork fat for 14 days | moderate |
| 49 | 181 | Pork fat for 14 days | moderate |
| 50 | 60 | Egg yolk for 20 hours | moderate |
| 51 | 169 | Egg yolk for 20 hours | large |
| 52 | 81 | Egg yolk for 20 hours | large |
| 53 | 114 | Egg yolk for 36 hours | large |
| 54 | 209 | Egg yolk for 36 hours | moderate |
| 55 | 78 | Egg yolk for 6 days | moderate |
| 56 | 234 | Egg yolk for 14 days | large |
| 57 | 193 | Egg yolk for 14 days | moderate |
| 58 | 101 | Egg yolk for 14 days | moderate |
| 59 | 164 | Egg yolk for 14 days | large |

four individual rats of this group were fed as follows: four, olive oil; seven, pork fat; ten, egg yolk; thirteen, butter. Fifteen individuals of the group have a moderate amount of fat in the myocardial fibers, as in figure 3, while nineteen have a large amount as in figure 4. The cardiac fibers of the rats in this group contain an unusual amount of neutral fat due no doubt to the fatty character of the food. The animals would eat but sparingly of olive oil and the effect produced was less marked than in the case of butter and pork fat which were eagerly consumed. A number of rats (nos. 48, 49, 57, 58) would eat but little fat after the first few days and the fat content of the myocardial fibers was no more than in animals living on carbohydrates and protein. Figure 4 represents a transverse section of ventricular fibers from a rat (no. 46) which was killed twenty hours after consuming eight or ten grams of pork fat. The cardiac fibers are loaded with droplets. As will be seen from table 3 the large amount of fat makes its appearance in the heart with astonishing rapidity, reaching a maximum in from twelve to twenty-four hours after but one or two large feedings of a fatty food. Even when a fatty diet is continued for as long as twelve or fourteen days the amount of fat in the cardiac fibers is no more than after a single large fatty meal, although the animal may show a marked increase of adipose tissue.

Figure 4 may be taken as representative of the fat fed group, just as figure 1 was considered representative of the inanition group and figure 3 of the normally fed group. It is quite clear that the cardiac muscle fibers of the rat normally contain a very considerable quantity of fat in a microscopically visible form. Also in inanition the normal quantity is diminished almost to the point of complete disappearance while in animals on a fatty diet the cardiac fibers are usually loaded with droplets.

Cats. Table 4 shows a group of normal cats which were fed for three to ten days on a well balanced ration of bread, milk and moderately fat boiled beef. Of the twenty animals in this group eleven have a moderate amount of fat in the cardiac fibers (similar to fig. 3), six have a large amount (similar to fig. 4),

TABLE 4

Cats, fed three to ten days on wheat bread, whole milk and moderately fat boiled beef

| ANIMAL NUMBER | WEIGHT IN GRAMS WHEN KILLED | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) SMALL AS IN FIG. 2 (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) (VERY LARGE AS IN FIG. 5) | AMOUNT OF FAT IN MUSCLE FIBERS OF ITS BUNDLE (LEFT LIMB) |
|---------------|-----------------------------------|---|--|
| 60 | 332 | large | large |
| 61 | 337 | large | moderate |
| 62 | 368 | moderate | large |
| 63 | 375 | very large | very large |
| 64 | 416 | moderate | very large |
| 65 | 445 | moderate | moderate |
| 66 | 504 | large | very large |
| 67 | 525 | moderate | moderate |
| 68 | 592 | moderate | large |
| 69 | 1054 | moderate | large |
| 70 | 1376 | large | very large |
| 71 | 1664 | large | large |
| 72 | 1830 | large | moderate |
| 73 | 2206 | moderate | moderate |
| 74 | 2235 | small | moderate |
| 75 | 2245 | moderate | moderate |
| 76 | 2364 | moderate | large |
| 77 | 2432 | moderate | moderate |
| 78 | 2750 | moderate | moderate |
| 79 | 2816 | small | small |

two have a small amount (similar to fig. 2) and one has a very large amount (similar to fig. 6). It is evident that the myocardial fibers of normal cats contain fat in visible form and to an extent exceeding that found in rats. After ninety-six hours without food, rats are usually in a state of extreme inanition and show very few droplets in the cardiac fibers (table 3) but the same does not hold for cats. Cats (nos. 80 and 83, table 5) kept for ninety-six hours without food still show a moderate amount of fat in the heart. As is well known cats reach the last stages of inanition only after having been kept for about three weeks without food. For the purposes of this investigation, I have thought it unnecessary to subject cats to a long period of starvation. My results show only that during a fast of three or four days fat does not disappear from the cat heart. It is very probable that in the

last stages of inanition the cardiac fibers of the cat, like those of the rat, would contain little fat.

TABLE 5
Cats, fed as indicated

| ANIMAL NUMBER | WEIGHT IN GRAMS WHEN KILLED | FOOD GIVEN | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) (VERY LARGE AS IN FIG. 6) | AMOUNT OF FAT IN MUSCLE FIBERS OF HIS BUNDLE (LEFT LIMB) |
|---------------|-----------------------------|--|---|--|
| 80 | 465 | No food for 96 hours | moderate | moderate |
| 81 | 620 | 4 day fast, given butter, killed after 20 hours | very large | very large |
| 82 | 576 | 3 day fast, given pork fat 15 grams, killed after 20 hours. | very large | large |
| 83 | 666 | No food for 96 hours | large | large |
| 84 | 810 | 3 day fast, given pork fat large meal, killed after 17 hours | very large | moderate, fig. 6 |
| 85 | 885 | 3 day fast, fed 7 gms. butter, killed after 17 hours | moderate | small |
| 86 | 1137 | Bread and water 7 days | moderate | moderate |
| 87 | 1194 | Pork fat 1 day | large | large |
| 88 | 1259 | Bread and water 10 days | moderate | moderate |
| 89 | 1285 | Bread and water 10 days | moderate | small |

TABLE 6
Dogs, killed as soon as brought to the laboratory, animals in good nutritive condition, no special feeding

| ANIMAL NUMBER | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (SMALL AS IN FIG. 2) (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) | AMOUNT OF FAT IN MUSCLE FIBERS OF HIS BUNDLE (LEFT LIMB) |
|---------------|--|--|
| 90 | moderate | small, fig. 10 |
| 91 | small | very small |
| 92 | very small | very small |
| 93 | moderate | moderate |
| 94 | large | moderate |
| 95 | small | very small |

Dogs. In table 6 are listed six apparently normal dogs which were killed almost as soon as brought to the laboratory and without any special feeding. Two of the group have a moderate amount of fat in the cardiac fibers as in figure 10 at D and L; one has a large amount (similar to fig. 4); two have a small amount (similar to fig. 2); one has a very small amount (similar to fig. 1).

Fattened cattle and hogs. It may be doubted if cattle and hogs fattened for slaughter are to be regarded as normal. Admitting

TABLE 7
*Adult hogs, sheep and oxen fattened
for slaughter*

| ANIMAL AND NUMBER | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (VERY SMALL AS IN FIG. 1) (SMALL AS IN FIG. 2) (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) | AMOUNT OF FAT IN MUSCLE FIBERS OF HIS BUNDLE (LEFT LIMB) |
|-------------------|---|--|
| 96 Hog... | moderate | very small |
| 97 Hog... | moderate | very small |
| 98 Hog... | large | small |
| 99 Hog... | moderate | very small |
| 100 Hog... | small | very small |
| 101 Hog... | moderate | small |
| 102 Hog... | very small | very small |
| 103 Hog... | large | small |
| 104 Hog... | large | very small |
| 105 Hog... | small | very small |
| 106 Sheep.. | very large | very small |
| 107 Sheep.. | large | small |
| 108 Sheep.. | small | very small |
| 109 Sheep.. | moderate | very small |
| 110 Sheep.. | large | small |
| 111 Sheep.. | large | small |
| 112 Sheep.. | moderate | very small |
| 113 Sheep.. | moderate | very small |
| 114 Ox.... | large | very small |
| 115 Ox.... | large | very small |
| 116 Ox... | large | small |
| 117 Ox.... | moderate | very small |

that they are not strictly normal one should bear in mind that they are not markedly pathological and in nearly all cases they would soon return to the normal if over-feeding were discontinued. Table 7 shows the results obtained by the examination of the hearts of ten hogs, eight sheep and four oxen. The amount of fat in the cardiac fibers of the ox and sheep appears to be on the average somewhat greater than in hogs although the latter animals show more subcutaneous adipose tissue. Figure 9, at L and D, shows a section of cardiac fibers from a hog (no. 104).

Fetuses and suckling animals. Table 8 is intended to give an idea of the amount of fat in the cardiac fibers during the later stages of fetal life and in sucklings. In the majority of cases shown in the table the quantity of fat is designated as moderate and in no case is it entirely absent. Figure 7 represents a section of muscle fibers from the apparently normal heart of an eight months human fetus.

Other animals. The heart muscle of several of each of the following species was examined; mouse, opossum, rabbit, guinea-pig, monkey. In each of these animals a considerable quantity of visible fat is normally present in the cardiac fibers.

Human. Excepting in comparatively rare cases of sudden and violent death, human hearts at autopsy are seldom to be considered strictly normal. In many cases death is preceded by a period of inanition and as we have seen, this condition may be expected to bring about the more or less complete disappearance of visible fat from the cardiac fibers. In several human hearts, however, which were normal in color and showed no cloudiness, opacity or yellowish white appearance, a moderate amount of fat was present in the typical diffuse general form found in the normal hearts of animals. I believe that it will finally be shown beyond doubt that microscopically visible fat is present in normal human cardiac muscle. This view is advocated by Bell ('12) and Wegelin ('13) each of whom has demonstrated visible fat in apparently normal human hearts. There can be little doubt, however, that the well known 'mottled fatty degeneration' is pathological.

TABLE 8

Suckling animals, fetuses, two young children

| ANIMAL AND NUMBER | AMOUNT OF FAT IN HEART MUSCLE (LEFT VENTRICLE) (VERY SMALL AS IN FIG. 1) (SMALL AS IN FIG. 2) (MODERATE AS IN FIG. 3) (LARGE AS IN FIG. 4) | AMOUNT OF FAT IN MUSCLE FIBERS OF HIS BUNDLE (LEFT LIMB) |
|--|---|--|
| | | |
| 118 Suckling kitten, 195 grams..... | large | large |
| 119 Suckling kitten, 209 grams..... | moderate | moderate |
| 120 Suckling kitten, 226 grams..... | moderate | small |
| 121-124 inclusive. Suckling albino rats, various ages..... | moderate | |
| 125-130 inclusive, dog fetuses from 193 -273 grams, all from one litter.. | moderate | moderate or small |
| 131-133 inclusive, pig fetuses nearly at term..... | moderate to small | |
| 134 fetal calf, 35 cm..... | moderate | |
| 135 fetal calf, 40 cm..... | small | |
| 136 fetal calf, full term..... | moderate | |
| 137 fetal calf, full term..... | moderate | |
| 138 5 mos. human fetus..... | moderate | small |
| 139 7 mos. human fetus..... | moderate | moderate |
| 140 7 mos. human fetus..... | moderate | small |
| 141 8 mos. human fetus..... | moderate | moderate, fig. 7 |
| 142 8 mos. human fetus..... | small | moderate |
| 143 Child 11 mos. old (congenital- syphilis)..... | small | very small |
| 144 child 3 years old (empyema)..... | moderate | large |

PHYSIOLOGICAL SIGNIFICANCE OF NEUTRAL FAT IN CARDIAC MUSCLE

From the experimental observations just recorded the conclusion seems justified that the appearance of visible fat in cardiac muscle fibers is not *per se* to be regarded as evidence of disturbed cell metabolism or any other pathological change. Under normal physiological conditions the myocardial fibers of rats contain a somewhat variable, but usually moderate, amount of neutral fat in the form of droplets (fig. 3). When the animal is starved the quantity of fat in the heart muscle is markedly diminished even to complete disappearance (fig. 1). When the animal is fed

upon fats the number and size of droplets in the cardiac fibers is, within a few hours, increased to such an extent as to present a picture which some would describe as marked fatty degeneration (fig. 4). There is, however, no evidence of degenerative change. The same general conditions no doubt apply to all laboratory mammals.

Either directly or by implication I have here offered the following reasons in support of the idea that visible droplets of neutral fat are of physiological occurrence in the cardiac muscle fibers of mammals: 1) In each of several well known species here studied visible fat is found with great regularity in the heart muscle of all individuals whether fetal, young or adult. Very few, if any, of the cardiac fibers are absolutely fat free. 2) The fat droplets are not found in the contractile elements. They occupy a definite position in the sarcoplasm and do not interrupt the continuity of Krause's membranes. 3) There are found in the muscle fibers neither degenerative changes nor other evidence of any pathological alteration of structure. 4) There is no evidence of functional disturbance of the cardiac muscle. 5) The quantity of fat is variable, is decreased in inanition and increased by fatty foods. 6) Fat in cardiac muscle, with respect to general occurrence, cytological relations and relation to nutrition, is closely similar to that in skeletal muscle. An increasing number of observers now regard the occurrence of visible fat in the latter type of muscle as normal.

It is well established that microscopically visible fat in cardiac muscle is sometimes pathological. The mottled 'degeneration' causing the well known tiger lily, tabby cat or thrush breast appearance is an example. Those who insist that microscopically visible fat in the heart is invariably of pathological significance base their claim not so much upon any observed derangement of cell function or degenerative change in cell protoplasm as upon the assumption that normally the intake of fat is at all times exactly balanced by utilization. Does this balance actually exist? Until recently such a balance was assumed in all the parenchymatous cells of the body, those of adipose tissue and the suprarenal alone being exceptions. It is now well known that

few if any of the organs of the body are chemically fat free and that visible fat is normally present in many of the parenchymatous cells including nearly all of the gland cells and the skeletal muscle fibers. In these active tissues there is normally a variable quantity of visible fat which represents the reserve between intake and utilization. The experimental observations here recorded clearly indicate that in cardiac muscle fibers a certain visible reserve of fat is normal and this reserve may at times appear surprisingly large without pathological significance. In reality the fat reserve, in proportion to the great amount of work done by the heart, is at all times very small. Under normal conditions the sugar of the blood, often stored as glycogen in cardiac muscle, is known to be the chief source of the energy of the heart beat.

OCCURRENCE AND SIGNIFICANCE OF NEUTRAL FAT IN THE MUSCLE TISSUE OF THE ATRIO-VENTRICULAR SYSTEM

Fatty degeneration of the muscle fibers of the His bundle has been reported in fatal cases of diphtheria by Amenomiya ('10), Sternberg ('10), Monrad-Krohn ('11), and Tanaka ('12). Mönckeberg ('08) definitely rejects the possibility that visible fat in the Purkinje fibers may be normal, and advances the idea that fatty degeneration of the His bundle is frequently 'the anatomical expression of the immediate cause of death.' Engel ('10) who examined eighty-nine human hearts has made a more detailed investigation of this question than any yet attempted. In the human fetus, in children, and in young individuals she found no fat in the Purkinje fibers but in individuals past forty years of age the fibers invariably contained fat. Engel believes the fatty condition somewhat pathological, but considers it of slight clinical significance since the function of the conductive system is not impaired. Aschoff ('10) holds similar views.

It is well-known that the muscle tissue of the atrio-ventricular system has a certain physiological independence, in part, due to an independent and abundant blood supply as well as an independent nerve supply. Mönckeberg ('08) has described 'fatty

degeneration' as involving in certain cases both the myocardium proper and the His bundle, in other cases he finds droplets only in the cardiac fibers, in still others the bundle alone is affected. According to Engel ('10) there is usually more fat in the fibers of the bundle than in the myocardium proper. Sternberg ('10) has been able to observe no difference in this respect while Monrad-Krohn ('11) has found the heart muscle invariably more fatty than the Purkinje fibers.

In the literature I have found but a single reference to an examination for fat of the atrio-ventricular system in the lower animals. Engel ('10) reports that she was unable to demonstrate any fat in the Purkinje fibers of the pig and sheep. Most of the observers just cited have examined frozen sections, from the interventricular septum, a short distance below the aortic valve, showing both the myocardium and the left limb of the bundle. With respect to the fat content of the Purkinje fibers such sections may usually be regarded as typical of the bundle as a whole. The observations recorded in tables 4, 5, 6, 7 and 8, have reference to sections from the region just indicated. I have also frequently examined sections of the right limb and of the end branchings of both limbs. In a few cases the muscle fibers of the sino-auricular node and of the atrio-ventricular node were also examined. I have not studied the bundle of His in rats for the reason that it is small and in frozen sections it is frequently difficult to distinguish the muscle fibers of the bundle from the cardiac fibers. With other animals this difficulty was not experienced.

Sheep, hog and ox. Figure 9 represents a transverse section from the moderator band of a hog (no. 104, table 7). The Purkinje fibers, P, contain a much smaller amount of fat than the cardiac fibers, D and L. In the sheep, hog and ox, as is well known, the fibers of the atrio-ventricular system are of the typical Purkinje type and morphologically they differ widely from ordinary cardiac muscle. In these animals (table 7) the Purkinje fibers and muscle tissue of the atrio-ventricular node and of the sino-auricular node, contain normally but a very small amount of fat. As shown in the tables, this is the case even

when the cardiac muscle of an individual contains a large fat deposit. In the papillary muscles of the sheep's heart I have several times been able to trace the transition from Purkinje fibers to typical cardiac fibers. On passing from the former type to the latter the fibers become increasingly fatty. The droplets in the Purkinje fibers are frequently most numerous in the sarcoplasm immediately surrounding the nuclei, or they may be scattered throughout the fibers or may occur in groups or clusters.

Dogs. Figure 10 shows a section from the left limb of the bundle in a dog (no. 90, table 6). Cardiac fibers are marked D and L, Purkinje fibers, P. A section of the atrio-ventricular node of the same heart is shown in figure 12 and of the sino-auricular node in figure 11. In the hearts of the six dogs listed in table 6 the Purkinje fibers contain somewhat less fat than the cardiac fibers but more than the fibers of the nodal tissue.

Cats. In figure 6 at P are seen a few fibers from the left limb of the His bundle of a cat (no. 84, table 3). Cardiac fibers are shown at C. The amount of fat in the Purkinje fibers in this individual is less than in the cardiac fibers. Of the thirty cats listed in tables 4 and 5, eight have more fat in Purkinje fibers than in cardiac fibers, six have more in cardiac fibers, while in sixteen individuals the amount in the two systems is approximately equal. I have not examined the nodal tissue of the heart in cats.

Human. Figure 7 shows fat in the cardiac fibers and His bundle of an eight months human fetus. Table 8 gives an idea of the fat content of the Purkinje fibers of five human fetuses. Two children, one of eleven months (congenital syphilis) and one of three years (empyema) are also listed in the table. I have examined the conductive system in only six adult human hearts. Fat was found in the muscle fibers of the bundle in each case and in two individuals the amount was very large. Figure 8 represents a section from the interventricular septum of the heart in a fatal case of lobar pneumonia occurring in a negress aged fifty-three. No doubt the conditions represented in the figure would usually be regarded as decidedly pathological. From the observations of Engel ('10) and Aschoff ('10), however,

it would appear to be of little or no clinical significance. In several of the normal cats listed in tables 4 and 5 the amount of fat in the Purkinje fibers was nearly as large as in this case of pneumonia.

Differences due to species. In sheep, hogs and oxen the bundle of His is composed of typical Purkinje fibers and these normally contain only a small amount of fat even when the cardiac fibers are crowded with droplets. In cats, dogs and in man, as is well known, the Purkinje fibers are not typical but resemble more closely the ordinary cardiac muscle. In these latter animals the amount of fat in the muscle fibers of the bundle of His varies between wide limits and is often as much or more than is found in the cardiac fibers. In both groups of animals the muscle fibers of the sino-auricular node and the atrio-ventricular node normally contain only a small quantity of fat.

Physiological significance. From the above observations it is evident that visible fat droplets are normally and almost invariably present in the muscle fibers of the His bundle both in the fetus and in adults. It would be going too far to say that fatty droplets in the muscle fibers of the atrio-ventricular system are never of pathological significance. My observations are not sufficiently extensive to justify any conclusion regarding the relation between nutrition and fat in the Purkinje fibers.

INTERSTITIAL GRANULES (MITOCHONDRIA) AND PHOSPHOLIPINES IN CARDIAC MUSCLE

The phospholipine content (lecithine and related compounds) of cardiac muscle is, I believe, to be found in association with a non-fatty substance in the true interstitial granules of Kölliker ('89). Knoll ('91) long ago pointed out that these granules swell in water and stain with gold chloride and concluded that they may be composed, in part, of lecithine.

Figures 15 and 16 show the true interstitial granules in the cardiac muscle fibers of a dog. The sections were stained by Bensley's acid-fuchsin toluidin blue method as given by Cowdry ('12). Similar granules were observed in the cardiac fibers of the other animals used in this study. True interstitial granules are known

to occur in skeletal muscle and have been described in cardiac muscle by Holmgren ('10), Regaud ('09) and Prenant ('11). In most investigations concerning the structure and physiology of muscle, particularly of cardiac muscle, these granules are ignored. At the present time we possess little definite knowledge of their chemical nature and still less of their physiological significance. According to Regaud ('09) they are mitochondria and chemically an albuminlipoid compound or mixture.

The true interstitial granules are by no means identical with the structures which I have described as neutral fat droplets. The properties both chemical and morphological of each type mark it off sharply from the other and I have not been able to observe any transitional forms.

True interstitial granules appear to be composed of a rather soft plastic substance which easily takes the form imposed by surrounding structures. They are seen as threads, rodules, flattened plates, dumbbell shaped bodies, diplosomes, ovoids and rarely as spheres. In cardiac muscle these granules are usually limited to segment Q and this with great precision, figure 15.

In order to determine the chemical nature of the true interstitial granules I have made a study of numerous preparations both fresh, fixed, stained and unstained. As regards refractive index the granules differ but little from the myo-fibrillae. It is therefore difficult, although at times not impossible, to observe them in fresh unstained tissue. I have not determined with certainty whether they are singly or doubly refractive but it is certain that they are not typical doubly refractive fluid crystals of cholesterin ester. In fresh preparations mounted in water one can observe that the granules give rise to forms identical with or closely resembling myelin figures which stain with cresyl violet and at times with neutral red. With Scharlach R. the granules stain but faintly or not at all. In fresh unfixed tissue they are easily stained by dilute aqueous solutions of cresyl violet (Krause '11) but after treatment with absolute alcohol or other fat solvents they no longer take the stain. They are readily stained by the so-called mitochondrial methods but when the tissues are placed in absolute alcohol before being chromated the granules appear as

poorly stained shrunken fragments, having evidently suffered a loss of substance. This shows that the granules contain a non-fatty substance together with a substance soluble in fat solvents but rendered insoluble by the action of potassium bichromate. The shrinkage of the granules in absolute alcohol is evidently not due to dehydration for the substance which is removed from them is stained by basic dyes, and is rendered insoluble by potassium bichromate. This makes it certain that the substance removed by alcohol is not aqueous but fatty.

From the above observations we may conclude that the true interstitial granules of cardiac fibers are made up of at least two substances, one fatty the other non-fatty. The properties of the fatty component indicate that it can not be neutral fat but may very well be a phospholipine. Remembering that neutral fats and phospholipines together are known to constitute practically the entire fat content of cardiac muscle, the fatty substance of the granules, since it does not correspond to the former must be the latter—phospholipine. We have, moreover, already accounted for the neutral fat in the form of droplets which stain with Herxheimer's Scharlach R.

The conclusions just drawn are in full accord with the chemical findings of Krehl ('93) and Rubow ('04-'05) who have shown by analyses that the phospholipine content of heart muscle is remarkably uniform, being well-nigh constant even in inanition. The neutral fat, on the other hand, fluctuates in amount. The true interstitial granules under all conditions yet observed are surprisingly uniform and not subject to any definite variation even in inanition while the quantity of Scharlach R. fat is variable. Erlandsen ('07) demonstrated that cardiac muscle contains more than twice the quantity of phospholipine that is present in skeletal muscle. We again find that the true interstitial granules are, on the average, more than twice as abundant in cardiac as in skeletal muscle. Those who are familiar with the staining reactions and microchemical properties of mitochondria are no doubt aware that the properties of the granules, as given above, indicate that the latter structures are in fact mitochondria as was stated by Regaud ('09).

One may form some idea of the quantity of the phospholipines in the fibers by observing the number and size of the true interstitial granules and by taking into consideration also the extent to which they suffer loss of substance when treated with a fat solvent. Estimated in this rough manner the quantity of phospholipine in the granules appears to be sufficient to account for the total amount of the one to two per cent by weight of this substance shown by chemical analyses.

According to Kölliker ('89), Schaffer ('93), Altmann ('94), Wegelin ('13) and others, true interstitial granules, under either pathological or physiological conditions give origin to fat droplets either by fatty metamorphosis or by serving as foci under the influence of which neutral fat is deposited or synthesized. All the evidence which I have so far been able to obtain is directly opposed to this view.

If the true interstitial granules, as I have attempted to show, are composed in part of phospholipines the conception that they give rise to neutral fat may be taken, from a chemical standpoint, as equivalent to the formation of neutral fat from phospholipines (lecithine and related compounds). Krehl ('93) and Rubow ('04-'05) after careful chemical analyses of normal and pathological hearts conclude that neutral fat is not formed from lecithine (phospholipine) and their results have met with general acceptance. On the other hand we are certain that the phospholipines of the body are built up from neutral fat. This occurs in the liver and perhaps in other organs (Leathes '10). If, as seems probable, it occurs in the heart one might well suppose that the true interstitial granules are actively concerned in the process. It is not improbable that a molecular supply of fatty acid, drawn either directly from the blood or from the neutral fat droplets of the fibers, furnishes the material from which the phospholipine of the granules is formed.

We know that certain of the phospholipines are optically active (Leathes '10). Figure 15 shows that the true interstitial granules of cardiac muscle are found in the anisotropic segment Q. This suggests the possibility that the anisotropic property of segment Q is dependent upon the presence of the phospholipines of the granules.

SUMMARY

The cardiac muscle fibers of mammals, both fetal and adult, normally contain a variable nutritive reserve in the form of droplets of neutral fat (figs. 2, 3, 4, 10, 13), and the existence in the heart of any considerable quantity of 'invisible' neutral fat is improbable.

Fat droplets of normal cardiac muscle are arranged in longitudinal and transverse rows in the sarcoplasm between the myofibrillae or muscle columns (fig. 5). Large droplets are in the Q band, smaller droplets in the J band.

Fibers which contain very little fat (light by transmitted light) may be found side by side with others which are crowded with droplets (dark by transmitted light) (figs. 3, 4, 5, 9, 10, 13). The occurrence of these two types of fibers in cardiac muscle is physiological and they correspond to the so-called light (non-fatty) and dark (fatty) fibers of skeletal muscle (fig. 14) which likewise are of normal occurrence.

In inanition the normal visible fat of cardiac muscle (fig. 2 or 3) is gradually decreased (fig. 1). When fatty foods are given a pronounced increase is seen as soon as digestion is complete (fig. 4).

The phospholipine (lecithine and related compounds) of cardiac muscle is found in the true interstitial granules (mitochondria) (figs. 15 and 16 at g.) and it is not markedly decreased in inanition nor increased when fats are given in the food. Neutral fat droplets in cardiac muscle do not arise from true interstitial granules.

Visible neutral fat is of normal occurrence in the muscle fibers of the bundle of His (figs. 6, 7, 9, 10 at P); only a small amount is found in the nodal tissue of the heart (figs. 11 and 12).

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PLATES

Magnification and optical equipment. Figures 1 to 7 inclusive and 10 to 14 inclusive, Leitz achromatic objective 6a, compensating ocular 6, $\times 500$. Figures 8 and 9 Leitz achromatic objective 4, compensating ocular 6, $\times 300$. Figures 15 and 16, Leitz apochromatic objective 2 mm., compensating ocular 6, $\times 1600$. All the figures were outlined with the camera lucida.

Staining and thickness of sections. Figures 1 to 14 inclusive are from frozen sections cut 30μ and stained with Herxheimer's Scharlach R. The fat droplets shown in these figures by no means include all those found in the thickness of the sections (30μ), but only such as fell within the depth of focus of the lens employed. Figures 15 and 16 are from sections 3μ thick stained by Bensley's acid-fuchsin toluidin blue (mitochondria) method.

ABBREVIATIONS

| | |
|--|--|
| <i>C</i> , cardiac muscle fiber | <i>G</i> , true interstitial granules (mitochondria) |
| <i>D</i> , dark cardiac muscle fiber | |
| <i>L</i> , light cardiac muscle fiber | <i>M</i> , muscle column |
| <i>P</i> , muscle fiber of bundle of His | <i>Z</i> , Krause's membrane |

PLATE 1

EXPLANATION OF FIGURES

1 Transverse section of cardiac muscle from the left ventricle of an albino rat kept without food for 96 hours (animal 23, table 2). The amount of fat in the muscle fibers is *very small*. $\times 500$.

2 The same as figure 1 from an albino rat receiving protein, carbohydrate and a small quantity of fat (animal 10, table 1). The amount of fat in cardiac fibers is *small*. $\times 500$.

3 The same as figures 1 and 2 from an albino rat fed as in figure 2 (animal 6, table 1). *Moderate* amount of fat in the muscle fibers; *L*, 'light' cardiac fibers; *D*, 'dark' cardiac fibers. $\times 500$.

4 The same as figures 1, 2 and 3 from an albino rat, killed twenty hours after consuming 8 or 10 grams of pork fat (animal 46, table 3). The amount of fat in the cardiac fibers is *large*; *L*, 'light' fiber; *D*, 'dark' fiber. $\times 500$.

5 Longitudinal section from same preparation as in figure 4; *L*, 'light' fibers; *D*, 'dark' fibers (animal 46, table 3). $\times 500$.

6 From the interventricular septum of a cat killed 17 hours after receiving a large meal of pork fat (animal 84, table 5). *Very large* amount of fat in cardiac fibers, *C*; *moderate or large* amount in Purkinje fibers of the left limb of bundle of His, *P*. $\times 500$.

7 From the interventricular septum of an eight months' human fetus (animal 141, table 8). There is a *moderate* amount of fat in the cardiac fibers, *C*, and also in the Purkinje fiber of the left limb of the bundle of His, *P*. $\times 500$.

8 From the interventricular septum of the heart of a woman aged 53, fatal lobar pneumonia, cardiac fibers, *C*, contain a *moderate* amount of fat; fibers of left limb of bundle of His, *P*, show a *very large* amount of fat. $\times 300$.

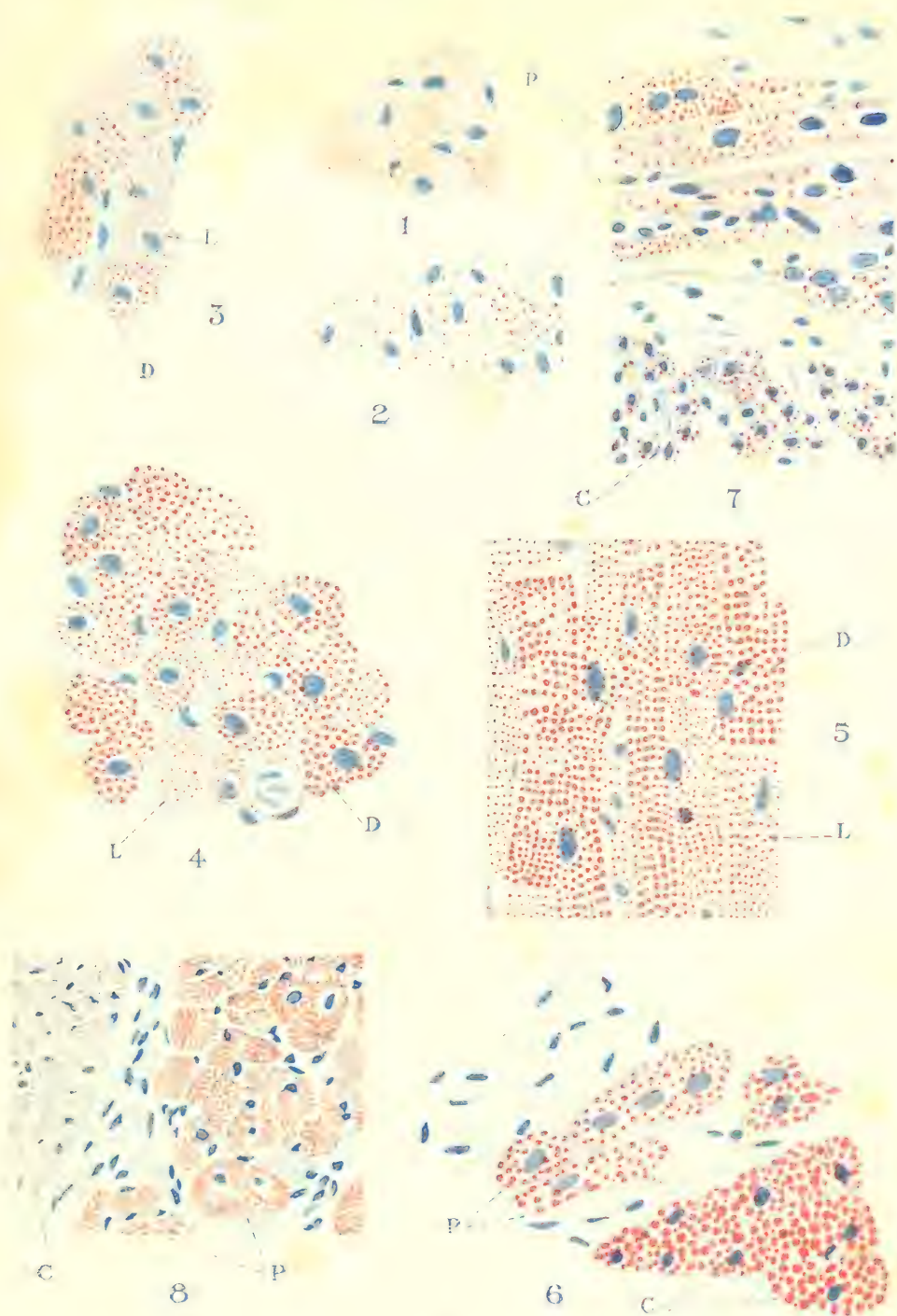


PLATE 2

EXPLANATION OF FIGURES

9 From the moderator band of a fattened hog (animal 104, table 7). Fibers of right limb of bundle of His, *P*, and light cardiac fibers, *L*, contain a *very small* amount of fat, dark cardiac fibers, *D*, a *large* amount. $\times 300$.

10 From the interventricular septum and left limb of the bundle of His of a well nourished dog (figs. 10 to 16 inclusive from same animal, no. 90, table 6). Dark cardiac fibers, *D*, and light cardiac fibers, *L*, contain a *moderate* amount of fat; Purkinje fibers, *P*, contain a *small* amount of fat. $\times 500$.

11 Muscle fibers from the sino-auricular node, heart of a normal dog (figs. 10 to 16 inclusive from same animal, no. 90, table 6). Fat content of muscle fibers *small*. $\times 500$.

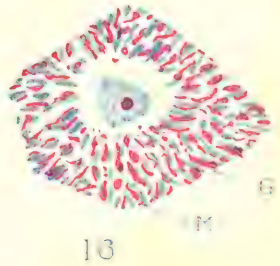
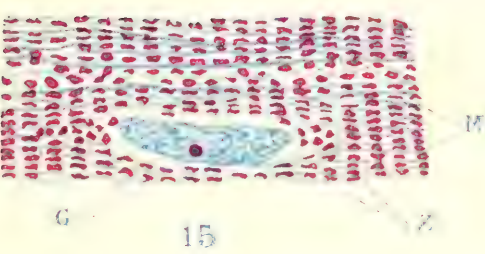
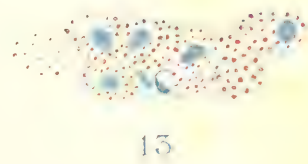
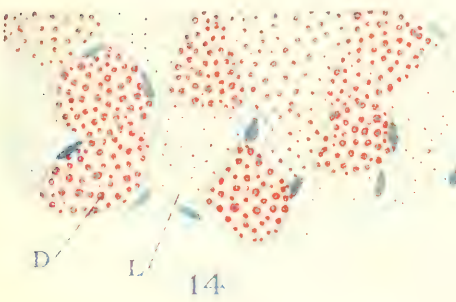
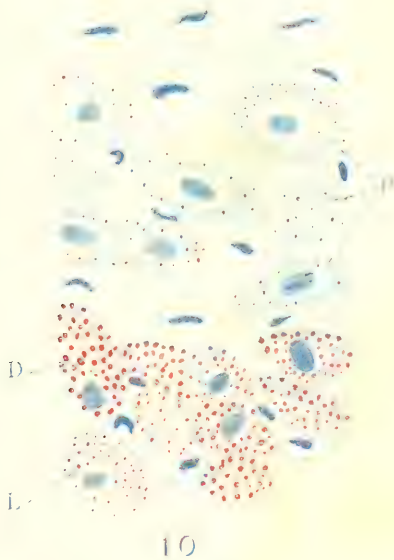
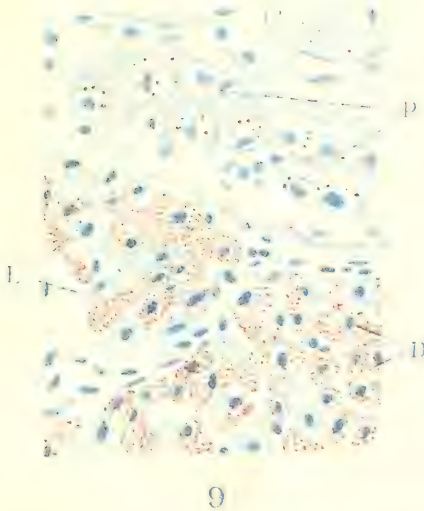
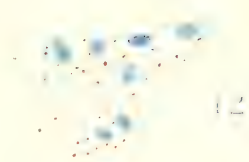
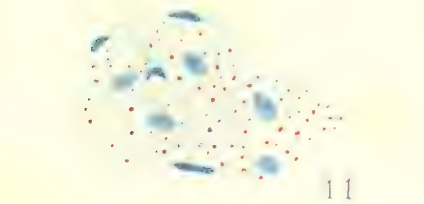
12 Muscle fibers from the atrio-ventricular node, heart of a normal dog (figs. 10 to 16 inclusive from same animal, no. 90, table 6). Fat content of muscle fibers *small*. $\times 500$.

13 Cardiac fibers from the right atrium of a normal dog (figs. 10 to 16 inclusive from same animal, no. 90, table 6), fat content *moderate*. $\times 500$.

14 Skeletal muscle fibers from the diaphragm of a normal dog (figs. 10 to 16 inclusive from same animal, no. 90, table 6), *large* amount of fat in dark fibers, *D*, and *small* amount in light fibers, *L*. $\times 500$.

15 Cardiac fiber from interventricular septum of a dog (fig. 10 to 16 inclusive from same animal, no. 90, table 6). True interstitial granules, *G* (mitochondria, sarcosomes, *Q* granules), muscle columns *M*, Krause's membranes *Z*. $\times 1600$.

16 Same as figure 15, transverse section. $\times 1600$.



THE NORMAL MODE OF SECRETION IN THE THYROID GLAND

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ONE PLATE IN COLOR

In the glands of the alimentary canal the process of secretion is associated with definite changes in the structure of the secreting cells, and with the accumulation in them of products, granular or otherwise, which may be interpreted as the organic antecedents of the secretion itself. Even in some of the internal secretory glands, as, for example, the islets of Langerhans of the pancreas, functioning is associated with the storage or exhaustion of intracellular products which may be similarly interpreted. By means of these secretion antecedents an observer, who has, by experiment and observation, acquainted himself with the secretory mode, may form an estimate of the secretory potential at the time of observation.

In the thyroid gland, on the other hand, the search for such evidences of secretory activity, has been, as regards the nature of the intracellular secretion antecedents, of so contradictory a nature, and of such doubtful functional import, that, at present, we are unable to state from the examination of a thyroid gland whether the gland was active or inactive. Accordingly, different observers, as, for example, in Grave's disease, in discussing the same results, have arrived at diametrically opposed conclusions.

One of the features of the thyroid gland, in particular, which baffled interpretation was the presence in it of a storage product, the so-called colloid, the route and rate of resorption of which have remained problematical, though chemical and physiological studies indicated that it contained the physiologically active

thyroid substances. Some observers have even doubted the resorption of this material, and have suggested that the function of the thyroid gland was primarily to withdraw toxic substances from the blood. Others have conceived the colloid as a sort of menstruum in which the real thyroid secretion was received and from which it might be withdrawn without visible change in the colloid itself. Still others have held the view that the colloid was the real secretion of the thyroid gland and that the normal mechanism of thyroid secretion was by this indirect route, first secreting into the centre of the follicle, and then withdrawing this ware-housed material, as functional needs required, by some unknown method and route.

The determination of the true significance of the colloid in the secretory cycle of the gland, and of the ways in which it is formed, and of its intracellular antecedents, is of fundamental importance in the physiology and pathology of the thyroid gland. The conviction that it is by this indirect method that the thyroid gland produces its internal secretion lies at the bottom of all of our more or less speculative interpretations of pathological conditions, and in view of the strong physiological evidence supporting this conviction few have had the courage to question its accuracy. Many authors have tried nevertheless to influence experimentally the rate of secretion in the gland, and to read in the changes so produced the true history of its secretory process. In this way many interesting facts have been discovered, which at present seem to some extent contradictory of one another, but which nevertheless must be found to be in accord when the true history of the process is revealed.

Our earliest knowledge as to the origin of the intrafollicular colloid of the thyroid gland is due to Biondi and Langendorff. Biondi ('89) showed that this substance was a true product of the secretory activity of the thyroid epithelial cells, inasmuch as he found globules of similarly staining substances in the cells themselves. He conceived the process of secretion as follows: the cells of the thyroid gland produce the colloid, since one can see in them little globules having the same microchemical reactions; the vesicle has a tendency to increase in size partly by

multiplication of the epithelial cells, partly by increase of the colloid; after filling itself the vesicle discharges into the nearest lymphatic vessel; finally the collapsed vesicle disposes itself in the form of a number of little acini which repeat the process.

Langendorff ('89) using the method of comparative study for the elucidation of the secretory process in the cells of the thyroid gland, reached conclusions which, in some respects, confirm and extend those of Biondi. He described two sorts of cells in the gland which he designated, respectively, principal cells, and colloid cells. The principal cells constituted the main mass of the epithelium. They were cylindrical or columnar cells, of variable height in different species and in different ages of the same animal species. They possessed a reticular protoplasm, with granules at the nodal points, and an oval or round nucleus situated at the basal end of the cell. Like Biondi he saw occasionally in these cells small hyaline spherules, but considered them to occur very rarely. The colloid cells differed from the principal cells by the hyaline, transparent appearance of their cytoplasm. This cytoplasm browned with osmic acid, and, in dyes, stained the same as the colloid content of the follicles. He found all grades of transition between the colloid cells and the principal cells. He regarded the colloid cells as elements engaged in the secretion of colloid but did not commit himself definitely to the opinion that, after a period of secretion, they might return to the state of the principal cells. He was likewise in doubt whether they degenerated or not after secretion.

V. Wyss ('89) studied the effects on the thyroid gland produced by poisoning with pilocarpine. He found in cats and dogs that the gland after pilocarpine was large, turgid, and filled with blood, and that the cells were larger, the nuclei less apparent. The free ends of the cells were prolonged into processes which were continuous with the colloid mass, and between these processes were brilliant spherules of apparently fluid nature.

Anderson ('94) confirmed V. Wyss' conclusions relative to the effect of pilocarpine on the gland, and studied the structure of the epithelial cells in young cats and rabbits at different periods of time after injections of pilocarpine. He described, in the

earlier phases of pilocarpinisation, the appearance of clear droplets in the cytoplasm, which collected at the free pole of the cell, to be extruded in the form of small droplets into the cavity of the vesicle. These, therefore, he regarded as the antecedents of the clear vacuoles of the margin of the colloid and on account of their lack of affinity for dyes, designated chromophobe secretion. A little later round, stainable droplets made their appearance in the free pole of the cell, which likewise migrated to the free border to be extruded into the lumen, constituting thus the chromophile secretion. He regarded the colloid cells of Langendorff as cells destined to degenerate. Thus, Anderson rejected the mode of secretion favored by Langendorff, and introduced the conception of a polyvalent secretion. His results, in general, are in more accord with those of Biondi than with those of Langendorff.

Hürthle ('94) in the same year studied the effects of reduction of thyroid tissue, and of bile retention, on the secretory processes of the gland. He found, as a result of each of these conditions, a great increase in the number of cells containing colloid spherules, which he therefore interpreted as an evidence of accelerated activity of the gland. On the other hand he recognized the occurrence of the Langendorff colloid cells to which he also ascribed secretory significance, and which he considered capable of transformation into principal cells.

Galeotti ('96) studied the thyroid glands of the turtle *Emys europaea*, under normal conditions, and after the injection of various products of metabolism. He described two sorts of secretion antecedents: fuchsinophile granules of nuclear origin, previously undescribed, and droplets of colloid like those described by Biondi, Anderson, and Hürthle. These two secretion antecedents varied independently of one another under the experimental conditions employed.

Following Galeotti a number of different observers using his methods studied the thyroid gland under different experimental and pathological conditions, confirming his results as to the double character of the thyroid secretion and the independent variation of the two sorts of secretion. Among these may be

mentioned Tiberti, and Ciulla. The latter identifies the fuchsinophile granulations of Galeotti with the chromophobe secretion of Anderson, the plasmosomes with the chromophile secretion of the same author.

Lobenhoffer ('09) studied human thyroid glands, both normal and pathological, in material fixed in formol Müller, and stained in anilin acid fuchsin. He found, in his preparations, the cells containing in widely varying amounts spherical fuchsinophile granules about the size of the granules of eosinophile leucocytes. Sometimes these granules formed a narrow row along the margin of the cell, and sometimes very small granules were found actually in the margin of the colloid. These granules he regarded as the antecedents of the thyroid secretion, interpreting the varying contents as indicating different phases of secretory activity.

As a result of the work of these observers we have to consider the following structures, in connection with the secretory activity of the thyroid cells, as possible intracellular secretion antecedents:

1. Spherules of colloid, described by all observers except Lobenhoffer.

2. Vacuoles containing a colorless unstaining fluid substance described by Anderson as chromophobe secretion, and interpreted by him as the antecedent of the content of the vacuoles seen in the margin of the colloid.

3. Colloid occurring as a diffusely distributed substance in the cytoplasm of the so-called colloid cells of Langendorff.

4. The fuchsinophile granules of Galeotti.

5. The fuchsinophile granules of Lobenhoffer.

Recent work by O. Schultze, and Mawas has helped to reduce the number of supposed secretion antecedents in the preceding list by demonstrating the presence in the thyroid epithelial cells of numerous mitochondria, usually filamentous, and oriented in the direction of the main axis of the cells. There seems to be little doubt that the fuchsinophile granules of Lobenhoffer are in reality mitochondria, rather imperfectly preserved. To this category belong also in part at least the fuchsinophile granules of Galeotti, Tiberti, and others. Possibly however, a part of these

granules are of another nature, since I have shown that in hyperplastic glands of the opossum and in human glands from cases of exophthalmic goiter, non-mitochondrial fuchsinophile granules occur. These will require further discussion when the secretory by-products are considered.

With the exception of Hürthle, Langendorff, and Schmidt, practically all observers agree that the colloid cells of Langendorff are cells in the last stages of cytomorphosis. The perfect gradation between these cells and the so-called principal cells on the one hand and the obviously degenerating cells of the follicle on the other hand leaves little doubt of their significance. The changes in the nucleus, the disappearance of mitochondria, and, in many cases, the visible disintegration of the cytoplasm, or desquamation of the cell, all point to the correctness of this conclusion.

Thus by elimination we arrive at the conclusion that the only secretory antecedents thus far demonstrated in the thyroid epithelial cells which may be considered to be normal products, are the colloid globules of Biondi, and Hürthle, to which belong also the chromophile granules of Anderson, and the so-called chromophobe secretion of Anderson. It is necessary therefore to examine in greater detail the occurrence of these products in the thyroid epithelial cells, with the object of determining whether they are actually related to the formation of intrafollicular colloid, whether they are sufficient to account for the physiological activity of the gland, and what indications they afford of the rate of formation of the intrafollicular colloid.

Hürthle found that, when the thyroid tissue was reduced by the removal of the whole of one lobe and two-thirds of the other, in many places in the gland the epithelial cells contained droplets of substance which was sharply defined by its staining reaction from the surrounding protoplasm, but agreed in all respects with the colloid contained in the follicular lumina. Similar results he obtained by ligating the common bile duct and the thoracic duct simultaneously. In these glands also he found the lymphatic vessels much dilated and filled with strongly staining colloid substance without admixture of formed elements. He con-

sidered two possible explanations of this phenomenon, namely, that it was due to lymphatic obstruction, and that it was due to accelerated activity of the gland, and decided in favor of the latter alternative because ligation of the thoracic duct alone produced no such changes in the gland.

Langendorff (*loc. cit.*) on the other hand, while admitting the occasional occurrence of colloid droplets in the cells, did not consider them of much significance from the secretory standpoint because of their extreme rarity. Anderson also saw them, not in the normal cell, but as a result of prolonged pilocarpinisation of the animal. Schmidt could find no effect on the structure of the epithelial cells as a result of pilocarpine injections, and attached more importance to the colloid cells as an indication of secretory activity. Bensley ('14) on the contrary, in studying the involution of the hyperplastic gland of the opossum produced by the administration of iodides, found that the cells of the gland practically all contained globules of colloid, and that they could be seen discharging it into the lumen, while colloid cells were almost completely lacking. In this case the restoration of the intrafollicular colloid was wholly by the formation of intracellular globules which discharged into the lumen. The process however was an extremely slow one; after seventeen days, though practically every cell contained a globule of colloid, as large as, or larger than the nucleus, there was little intrafollicular colloid, and at the end of twenty-four days of daily administration of iodides the condition was but slightly advanced; intracellular colloid remained about the same as in the preceding case but the follicular colloid was somewhat increased. Recent experiments on the hyperplastic glands of the opossum have amply confirmed these results; iodine administered daily to the animal with a hyperplastic gland produces a gradual involution marked by the slow accumulation in the cells of colloid droplets, usually a single drop to a cell, and the ultimate discharge of these into the newly formed lumen. We may consider it proven therefore that the production of colloid under certain special conditions is by this method.

This conclusion raises the question whether the production of colloid under normal conditions of functioning is by the same method, and, if so, what are the implications of this fact from the standpoint of secretory rate?

Hürthle claimed that the formation of colloid droplets in the epithelial cells of the thyroid gland was one of the ways of formation of colloid and that their presence was an indication of accelerated thyroid activity. Langendorff pointed out that they were extremely rare, and therefore could not have the secretory importance claimed by Hürthle. That Langendorff's contention in this respect is correct will readily be admitted. Indeed, one may search complete series of sections of small thyroid glands, and thousands of sections of larger ones without finding in them a single droplet of intracellular colloid. In six thyroid glands of man obtained at autopsies on executed criminals, and examined by the writer, only one contained epithelial cells with colloid droplets in them. In pathological glands from cases of exophthalmic goiter, simple colloid goiter, and colloid adenoma, on the other hand, they occurred with variable frequency. In the one normal gland that contained them the colloid drops occurred with great frequency. For the most part they were placed not at the free margin of the cell, but deep in the protoplasm, often alongside of the nucleus, and in many cases several drops formed a row extending from this deeper location to the free border. In many follicles, however, the colloid droplets occupied the tips of the epithelial cells, and in others the colloid masses inside the follicle could be seen to be made up of a cluster of small droplets, apparently derived from different cells, which had failed to fuse with one another inside of the follicle. This gland also contained an unusual number of colloid cells of Langendorff.

The obvious participation of these intracellular colloid droplets in the replenishment of the intrafollicular colloid, on the one hand, and the slowness of this process demonstrated by experiment, and the rarity of the occurrence of such droplets under normal conditions, on the other hand, suggest the following possibilities, which, however, are not, as will appear more clearly later, mutually exclusive: (1) the formation of colloid is an inter-

mittent function of the thyroid cells; (2) there are other, at present unknown mechanisms for the formation of colloid, correlated with droplet formation, but able to proceed without it; (3) the secretion of colloid into the gland lumen is an accessory and not the primary function of the epithelial cells of the gland.

For the reasons mentioned above, it is apparent that the formation of colloid droplets in the cell, at least, is an intermittent function. It is possible, however, that in addition to this mode of formation of colloid there is a slow and continuous production of colloid at the free margin of the cell unaccompanied by the formation of visible secretion antecedents in the cytoplasm, and it may be that this latter is the main method of production of intrafollicular colloid, the droplet method representing some upset of secretory equilibrium which results in the accumulation of the product of secretion in the cell, instead of the lumen. That this hypothetical upset is of the nature of an acceleration of the secretory rate is, however, excluded by the fact that we often see the droplet formation in the greatest abundance in adenomata the stroma of which is in an advanced state of hyaline degeneration, and in which, therefore, there can be no question of accelerated secretion rate. Histologically considered such a conception of the process of secretion in the thyroid gland must remain hypothetical, since it is incapable of objective proof.

The third possibility, namely, that the secretion of colloid into the gland lumen is an accessory and not the primary function of the epithelial cells, though correlated intimately with this primary function, would, if established, explain and include all of the facts. Such a theory to be accepted, must account for the irregular occurrence of droplets, for their formation in the interior of the cell rather than on either of the free surfaces, and for their increase under iodine or thyreoglobulin administration, and under the experimental conditions of Hürthle. It involves the assumption of a more remote antecedent of the secretion than the colloid droplets of Hürthle.

In my studies of the thyroid glands of various mammals, I have been struck with the frequent occurrence, particularly in the cat, dog and opossum, of vacuoles with unstainable con-

tents, rather irregular in shape, occurring in the base of the cell, and with the frequent occurrence in the cells of hyperplastic human glands from cases of true exophthalmic goiter of droplets of material staining like colloid located similarly in the extreme bases of the cell near the capillary net. Ferguson ('11), also, has described the occasional occurrence in the thyroid gland of elasmobranch fishes of cells presenting in their basal ends a ragged and rodded appearance which he interprets as due to secretion storage for direct export to the vascular channels.

A group of opossums kept under observation under various experimental conditions during the past winter have furnished material in which, by reason of the fact that these vacuolar substances in the cell were unusually increased in amount, it was possible to study their variation and to develop a technique for staining of their contents. One group of these animals was kept for a period of three weeks on a dietary consisting of beef, bread and fat, egg, bread and fat, or cheese, bread and fat, just sufficient to maintain constant weight. In another group the diet was so regulated that with constant bread and fat content there was a progressive increment of meat fed to the successive members of the series. In all of the animals thus kept on a controlled diet, the thyroid cells contained such basal vacuoles, and in two of the animals of the second series, namely those which received, respectively, twice and two and a half times the normal meat ration, the material of this sort comprised fully half of the cell contents.

The fixation of the material is of considerable importance in the study of these vacuolar substances, because the contents are so dilute that they may be precipitated in an invisible form on the protoplasmic strands which wall the vacuoles. Formalin zenker, however, was found to precipitate the contents in the form of a thin gel sometimes filling completely the space of the vacuoles, sometimes containing small vacuoles from contraction in fixation. Staining however was difficult, because the material stained with the usual dyes in the same way as the protoplasm. With Mallory's connective tissue stain, however, it could be seen that the vacuoles had vaguely staining contents, but, since the

protoplasm also stained bluish after formalin zenker fixation, it was difficult to define accurately the limits of the vacuoles, and after fixation in ordinary Zenker's fluid the vacuoles did not stain at all. Accordingly the indication was to find some stable stain which would stain the protoplasm diffusely, and then to stain the secretion a contrast color. For this purpose brasilin in phosphotungstic acid solution was found to be effective. The solution is prepared as follows:

| | |
|----------------------------|-----------|
| Phosphotungstic acid | 1.0 g. |
| Distilled water..... | 100.0 cc. |
| Brasilin..... | 0.05 g. |

The brasilin is first dissolved in a small quantity of distilled water by the aid of heat and added to the phosphotungstic acid solution. Ripening may be accelerated by the addition of 0.4 cc. of hydrogen peroxide, or of a few drops of a solution of soluble molybdic acid. The solution deteriorates with age and should not be used after three days.

Sections of thyroid glands which have been fixed in formalin zenker, fastened to slides by the water method (if albumen is used it should be very small in amount) are passed through toluol, absolute alcohol, to water, iodised, and placed in the staining solution from one to several hours. The sections are then washed in water and placed for one to five minutes in the following solution:

| | |
|---------------------------|-----------|
| Phosphomolybdic acid..... | 1.0 g. |
| Wasserblau..... | 0.2 g. |
| Water..... | 100.0 cc. |

Then wash rapidly in water, dehydrate in absolute alcohol, clear in toluol, and mount in balsam.

In the preparations so stained with brasilin and wasserblau the cytoplasm stains pink to lilac, the nuclear chromatin, deep red, and the contents of the vacuoles sky blue, as shown in Fig. 1. The colloid droplets of Hürthle stain deep blue or deep red according to the concentration of the gel which composes them, which determines the diffusion rate of the dyes employed.

It must not be supposed that the technical difficulties of studying this intracellular product are wholly overcome by the method just described. When the material is large in amount the method is very satisfactory, but the intensity of the protoplasmic staining is not sufficient to define the material sharply when it is small in amount. Under these circumstances, a brief mordanting of the section, before staining, in a fresh solution of ammonium stannic chloride will improve the contrast staining, but will detract greatly from the transparency and beauty of the preparation.

The examination of the sections of the experimental series referred to above, and of a number of normal glands from animals killed as soon as obtained, reveals the presence in all, although in highly variable amounts in the individual members of the series, of a new secretion antecedent. This substance is in the form of vacuoles, occurring exclusively in the outer pole of the cell, which contain a dilute solution similar in its properties to the colloid of the follicular lumen, differing from the latter only in density. There are even in this substance clear vacuoles due to shrinkage in fixation, like those seen in the colloid of the lumen.

In two members of the experimental series, this substance is present in such amount that it fills quite half the cell. In these cases the cell presents an appearance comparable to that of the secreting cells of an exocrine gland like the pancreas, with the exception that the hylogens are in dilute solution in fairly large vacuoles instead of in the form of granules, and they are in the basal end of the cell instead of the free end. In other words these cells exhibit the ordinary picture of a secreting cell with stored secretion antecedents, but with reversed polarity.

Figure 1 shows an acinus from one of these glands. The cells are cylindrical in shape with a spherical nucleus placed rather nearer to the free end of the cell than to the base. The base of the cell is filled with sky-blue stained material contained in vacuoles separated from one another by thin sheets of cytoplasm containing mitochondrial filaments. The free pole of the cell directed towards the lumen is finely granular and stained a bluish-pink color. It contains none of the blue staining vacuolar

material, and consists solely of cytoplasm containing crowded mitochondrial filaments. In two cells of this figure small globules of colloid may be seen, in one case alongside of the nucleus, and in another in the apical cytoplasm.

In some of the thyroid glands obtained from opossums recently captured, consisting of fairly large follicles well filled with colloid, the epithelial cells appeared uniformly vacuolated, but when the preparations were stained with *brasilin* and *wasserblau* the vacuoles in the outer ends of the cells were found to be filled with blue staining material, those in the inner ends with unstainable material.

Three possible interpretations of the presence of this material suggest themselves: first, that it is a pathological product representing cytoplasmic degeneration, or imbibed serous fluid, or simple edema; second, that it is colloid in process of resorption by a transcellular route; third, that it is a true secretion antecedent representing material formed in the base of the cell for the purpose of direct transport into the vascular channels.

The fact that every cell of the gland contains the material, that it is present in some degree in all opossum thyroid glands, and that there are no other evidences of degeneration, such as changes in the nucleus or in the mitochondria, or in the intra-follicular connective tissue excludes the first possibility from consideration.

Opposed to the second of these hypotheses is the fact that only very exceptionally is this material found in the pole of the cell in contact with the follicular content, and then only when the cell is so loaded with the secretion that it is comparable in appearance to a parotid gland cell filled with zymogen granules. It might be possible to assume that the droplets of colloid occasionally seen in the cell are on the way out rather than proceeding towards the lumen. The evidence from the glands which are being reverted by iodine is, however, strictly opposed to this hypothesis, since a progressive increase in colloid in these cases has been demonstrated in an experimental series taken at different intervals of time and this increase is associated directly with colloid droplet formation and extrusion into the lumen. It is

conceivable, of course that the droplets of colloid are of two sorts, those destined for the follicular content, and those on their way to the base of the cell for secretion into the vascular channels of the gland. Opposed to this assumption is the fact that in entire glands, in the cells of which there is an abundance of the basal vacuolar substance there may be found not a single droplet of colloid of the dense type, and that the free poles of all the cells may be wholly free from products of secretion except where the crystals, of protein nature, demonstrated in a former article, project into this pole. Furthermore, in hyperplasia of long standing, in which there is practically no intrafollicular colloid, a large content of the new secretion in the form of small vacuoles distributed throughout the outer pole of the cell, may be present.

We are therefore forced to accept the third hypothesis which, physiologically considered, is the more attractive, inasmuch as it permits of harmonizing the various facts under a single hypothesis, namely that the secretion collected in the outer pole of the thyroid cell is destined to direct transport into the vascular channels, and that the thyroid cell represents a true reversal of polarity in accord with its endocrine function.

In addition to the facts mentioned above which point strongly to the correctness of this hypothesis, it may be pointed out that in exocrine glands fat droplets which are deposited in the secreting cells practically always make their appearance at the anti-secretory pole of the cell; this is the case in the pancreatic cells and in the chief cells of the gastric glands. The location of the fat deposits in the thyroid gland also is at the pole which according to the hypothesis here supported is the anti-secretory pole of the cell, namely the free end of the cell next the colloid.

We may assume therefore that the thyroid gland as all physiological and clinical experience indicates, prepares and secretes into the vascular channels of the gland a secretion, and that this secretion is formed in the outer pole of the cell, and excreted from it directly under normal conditions of functioning without passing by the indirect route through the follicular cavity.

It is necessary, however, under this hypothesis to explain the occurrence of intrafollicular colloid, and its variability in different members of a species and under different experimental conditions. In my recent studies on the changes in the hyperplastic gland of the opossum and its changes under domestication, I have shown that all the stored colloid may be withdrawn from the gland in a short period, and that the gland will maintain for a period of several months a condition in which little visible colloid is present in the gland, but, as Marine previously demonstrated in the dog, if iodine be administered the vesicles are reformed, and filled with dense colloid. In the opossum this colloid makes its appearance first deep in the thyroid cells, but migrates to the free surface and is there discharged into the lumen. I have also found in the study of many glands from cases of Basedow's disease that the few colloid droplets which are present are very frequently found at the level of the nucleus, or even in the base of the cell. These facts indicate that in addition to the direct mode of secretion there is an indirect mode, which consists in the condensation of the secretion into the form of droplets having a high content of solids, and the extrusion of these droplets into the follicular cavity. These droplets are formed in the same zone of the cell as that in which the primary or direct secretion is formed, and it is probable that they are formed at the expense of the latter.

The readiness with which the thyroid gland undergoes hyperplastic change, its responsiveness to iodine administration, as demonstrated by Marine and his co-workers, the ease by which it may be modified structurally by dietary conditions as shown by the work of Reid Hunt ('11), Marine, Chalmers Watson ('07), Tanberg ('00), and Missiroli ('10) and confirmed by my recent studies on the relation of diet to hyperplasia in opossums under domestication, confirm the conclusion that there is a delicate adjustment between the functioning of the thyroid gland and general body conditions, though at present we do not know the means by which this adjustment is mediated. This being the case it may be assumed that only when this adjustment is disturbed so that the rate of secretion is in excess of body needs, the indirect

mode of secretion comes in, and the product of secretion is condensed and stored in the intrafollicular cavity. It is conceivable, of course, that other factors than excess of production over functional needs might bring about this result, as, for example, an agent inhibiting direct export from the cell, or mechanical interference with the outflow from the cells. The latter influence is well illustrated in the colloid adenomata where, notwithstanding the fact that the stroma may be hyaline, the cells contain abundant colloid spherules, and thus give, according to the old criterion of Hürthle, the impression of high secretory activity. According to my hypothesis of thyroid secretion, this condition would represent a slow secretory activity of the epithelium of the tumor, all of the energy of which is, however, devoted to storage, since direct export by way of the vascular channels is impossible. This may explain the difference noted by Marine between the hyperplastic gland and the adenoma as to susceptibility to influence by iodine. The hyperplastic gland, whether its activity be high or low, is exporting its product directly. Iodine whether by accelerating the activity of the gland and so producing a condition of physiological saturation with thyroid products, or by actually inhibiting the export of material from the cell, causes the cell to reverse its processes, and store it in the follicular cavities. The adenoma is already storing all of the product which the vascular conditions and its specific cell equilibrium permit it to form, and so the process can not be influenced by iodine.

According to this conception of thyroid secretion the colloid in the thyroid vesicles is *per se* no measure of the activity of the gland at the moment of observation, though its consistence and its qualities may offer valuable indications of the capacity of the thyroid cell for normal storage. The colloid in fact may be the product of a storage phase which preceded the examination of the gland by a considerable period of time, since it is necessary to assume the resorption of this material only under the conditions where the normal direct secretory activity of the gland is insufficient to meet the functional demands. Accordingly, also, lack of colloid in the gland does not necessarily mean depression of the gland activity below the normal rate at the time of observation, though it probably does mean that there is such a

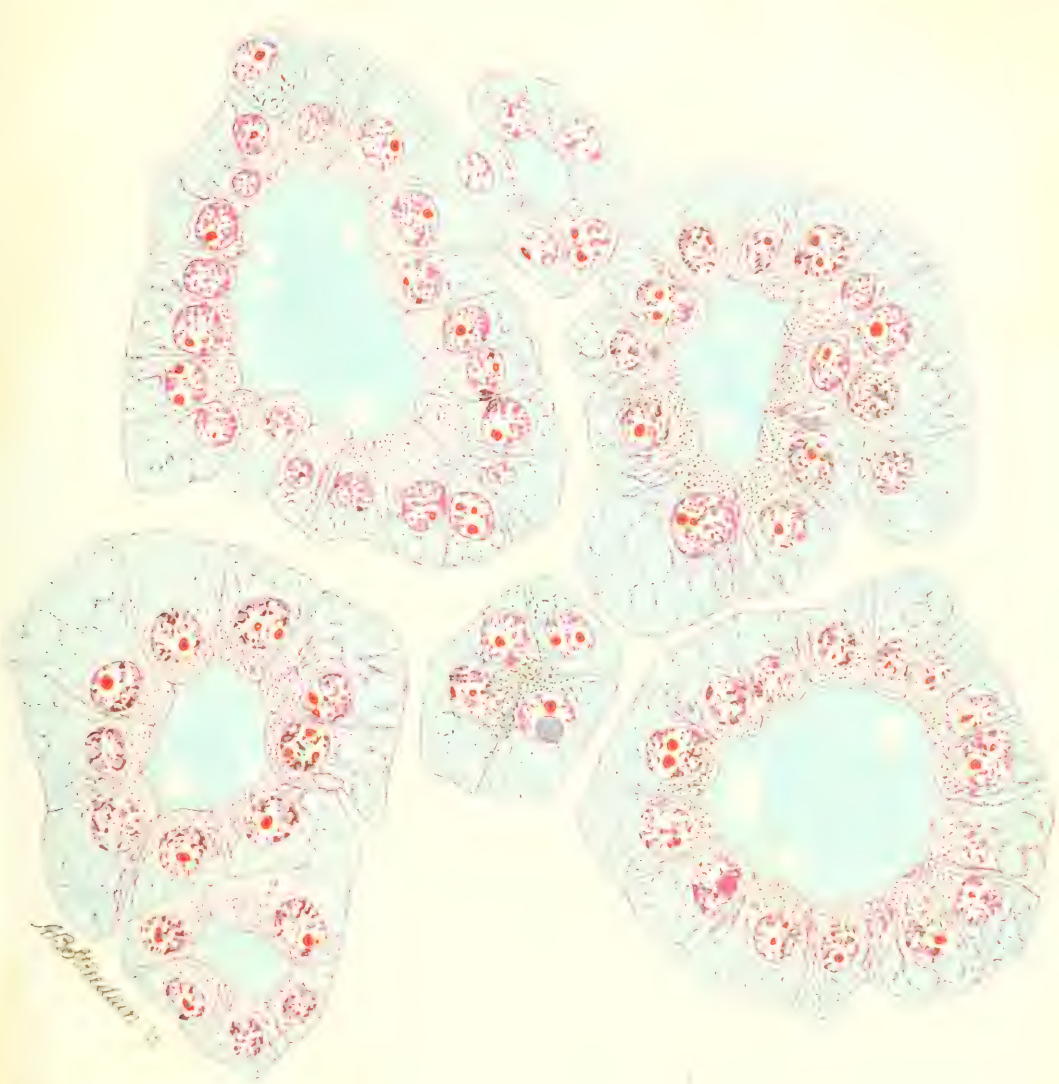
depression of physiological efficiency or has been at some previous time, and either that the gland has not risen above the level of secretory rate needed for direct export, or that there has been a failure of the normal mechanism of regulation. My observations on the hyperplastic glands of opossums by means of the methods described in this paper have shown that hyperplastic glands which appear almost identical histologically may yet differ markedly in the amount of these intracellular secretion antecedents which they contain, and thus, probably, in secretory potential.

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EXPLANATION OF PLATE

1 A group of follicles from the thyroid gland of the opossum, fixed in formalin zenker, stained with brasilin-wasserblau. $\times 1050$. In the outer poles of the cells a material differentially stained, similar to the intrafollicular colloid. In two cells droplets of colloid destined for storage.



THE INFLUENCE OF DIET AND IODIDES ON THE HYPERPLASIA OF THE THYROID GLAND OF OPOSSUMS IN CAPTIVITY

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In a recent paper on thyroid gland of the opossum ('14) I showed that, when these animals are brought under domestication, the thyroid gland undergoes a prompt and characteristic change, marked by hyperplasia of high degree, by disappearance of the stored colloid and of the intracellular crystals, and by the appearance in the free ends of the epithelial cells of granules which I interpreted as a new secretion antecedent. I showed also that this reaction was not a seasonal variation associated with hibernation, since animals captured at intervals throughout the winter months presented always a normal type of gland structure, while those kept for a few weeks in the laboratory, had hyperplastic glands. In one animal kept until June 1, also, there was no indication of spontaneous reversion as might be expected if the changes were associated with hibernation. On the contrary, reversion to a colloid type, as in Marine's experiments, was accomplished by the administration of iodine.

Since the reaction in question was obviously the result of new conditions incident to the confinement of the animals under laboratory conditions, it was apparent that the means for the control of this hyperplasia would also be available, if the factors operative in producing it could be discovered. Accordingly, I have devoted the animals which I have been able to secure this year to an attempt to determine the relations of iodine administration, and of dietary, to the changes described. While other factors, as, for example, injuries incident to capture, infections following such injuries, lack of exercise, mental conditions in captivity, and pathogenic organisms might conceivably be pri-

mary or contributing etiological factors in this condition, the work of Reid Hunt ('11), Chalmers Watson ('05), Missoroli ('13) and Marine ('14) on the relations of diet to hyperplasia in white rats and mice, and in the brook trout, and the potency of iodides in procuring reversion of hyperplastic glands to normal and in preventing hyperplasia demonstrated in many papers by Marine and his associates, pointed to diet and iodides as a subject for primary investigation.

In the former series of experiments the animals were kept confined in cages, and were fed on table scraps from the restaurant, that is to say on cooked food, containing abundance of meat, eggs, bread, etc. The animals under these conditions showed a pronounced preference for the meat and eggs. The food was renewed daily. Under these conditions the animals increased in weight rapidly, often in the course of three weeks nearly doubling their weights.

In the series of experiments which are reported in this paper, one animal of each group of experiments, was carried on the diet indicated above to see if the hyperplasia was produced and in what degree. The results as regards these controls are collected into table one. The remaining animals were divided into four groups, of which one group of two animals was used to determine whether iodides would revert the hyperplastic glands to a normal colloid type. A second group of three animals was kept under the same conditions as the controls except for the fact that they received from the day of their entry into the laboratory daily two drops of iodide of iron, to test whether iodine would inhibit the hyperplasia, if the factors producing it were present. A third group of eight animals was kept on dietaries designed to maintain them at nearly constant weight, and the fourth group of five animals was employed to test whether increasing the meat component of the diet would increase the reaction.

In every case, at the end of the experimental period, the animal was killed by bleeding from the femoral artery. The thyroid glands were removed as rapidly as possible and weighed, then fixed in formalin zenker, and acetic osmic bichromate.

Since the amount of colloid stored in the follicles of the gland varies within wide limits, even in normal glands, it was desirable to ascertain as nearly as possible the net weight of the gland tissue. This was accomplished, roughly, by drawing projected sections of the glands, cutting out the colloid areas, and weighing. The error in this method is, of course, considerable, but not nearly so great as the error introduced by neglecting the weight of the colloid itself in comparing the relative weights of animals and glands under experimental conditions. The unfortunate lack of any data on the normal curve of growth for these animals, such as those collected by Donaldson, Hatai and Jackson for the rat, increased the difficulty of comparison, but fortunately the differences obtained were for the most part of such magnitude, that little doubt remained of the nature of the changes in the experimental glands and of its direction, particularly in view of the fact that the histological examination of the gland confirmed the conclusions based on weight.

In the tables which follow the computation of the ratio of the gland weight—body weight has been made on the basis of the initial weight of the animal when received, since the fluctuations of weight were so great in some of the experiments, and since the experiments lasted too short a period in the majority of cases to allow for much natural growth.

Animals which were receiving iodides were placed in a special room in the basement, the rest of the colony in a room on the fifth floor, to avoid complicating the experiments by iodine in the atmosphere.

Table 1 includes the controls of the four succeeding series, that is, animals kept on an unrestricted diet of table scraps for different periods of time as indicated.

The magnitude of the increase in the weights of these glands will be better understood by comparing them with those of animals of similar weight in series four. Histologically all of these glands showed a high degree of hyperplasia, associated with reduction of the colloid in the follicles.

Number five which in the table shows a gain of weight amounting to only 480 grams at the end of the experiments, increased

TABLE 1

| NO. | SEX | INITIAL WEIGHT | CHANGE IN WEIGHT | THYROID GLAND | | | | DURATION OF EXPERIMENTS |
|-----|-----|----------------|------------------|---------------|------------|------------------|---------------------------|-------------------------|
| | | | | Gross weight | Net weight | Per cent colloid | Mgs. per kilo body weight | |
| 3 | F | 1350 | +1390 | 0.487 | 0.487 | 0 | 360.7 | 6 weeks |
| 4 | M | 3250 | +1990 | 0.353 | 0.3126 | 2.8 | 96.1 | 3 weeks |
| 5 | M | 2650 | + 480 | 0.461 | 0.4575 | 0.74 | 172.6 | 7½ weeks |
| 24 | M | 1760 | - 90 | 0.329 | 0.3165 | 3.78 | 179.8 | 12 weeks |

rapidly in weight during the first twenty days, reaching a weight of 4490 grams, an increase of 1840 grams. Then it began to lose weight and ten days later was reduced to 3960 grams. At this time the right lobe of the thyroid gland weighing 0.247 g. was removed, and the animal was placed on a diet which in series four had been found to keep the thyroid normal. The animal was killed twenty-two days later. The left thyroid weighed 0.214 g. but histologically showed no reversion to colloid type, or indeed any change.

Number twenty-four kept for twelve weeks on the mixed diet showed no gain in weight at any point, the weight fluctuating around the initial weight.

The animals of this series show an interesting gradation in thyroid activity correlated with the change in weight, as tested by the amount of intracellular secretion antecedent present in each case. In number three, in which the grade of hyperplasia was highest, and the percentage gain in weight greatest, the thyroid cells contained little secretion antecedent. Number four contained more secretion antecedent in the form of small vacuoles of stainable substance in the base of the cell. Number five contained a great deal of secretion antecedent in the form of small vacuoles of stainable substance in the outer pole of the cell, and the majority of the cells of number twenty-four contained still larger quantities of these substances, located in the bases of the cells.

These variations in secretory content have been tested by a new method of staining sections, fixed in formalin zenker, with phosphotungstic acid brasilin and wasserblau, which differen-

tiates an intracellular secretion antecedent in the form of vacuoles containing apparently a dilute solution of a substance, comparable to the colloid, but of less density, which stains blue with the wasserblau on a pink-stained protoplasmic background. They show that hyperplastic glands which ordinarily resemble one another very closely may nevertheless differ markedly in secretory potential as indicated by the amount of available antecedents in the cells. The results also show by the variability of the degree of hyperplasia, and of the secretory potential, that there are other factors influencing the rate of hyperplasia in these experiments, in addition to the dietary condition. The results, however, indicate clearly that hyperplasia of high degree follows these conditions, as in those described in the former paper.

Table 2 gives the quantitative results as regards the thyroid gland in two animals which, after a period of three weeks on an

TABLE 2

| NO. | SEX | INITIAL WEIGHT | CHANGE IN WEIGHT | THYROID GLAND | | | | DURATION OF EXPERIMENTS |
|-----|-----|----------------|------------------|---------------|------------|------------------|---------------------------|-------------------------|
| | | | | Gross weight | Net weight | Per cent colloid | Mgs. per kilo body weight | |
| 1 | M | 2400 | 1000 | 0.513 | 0.393 | 23.36 | 163.7 | 3-3 weeks |
| 2 | M | 2550 | 1500 | 0.710 | 0.5107 | 28.65 | 195 | 3-6 weeks |

unrestricted diet of table scraps, received two drops of syrup of iodide of iron daily, without change in the food. Although the animal which was kept for six weeks on iodides showed a higher degree of hyperplasia than the one kept for three weeks, it is not clear from this experiment whether the iodine checked the hyperplasia or not. The significant feature of this experiment is the enormous increase of colloid in the gland, confirming the former experiments on the opossum, and those of Marine on the dog as to the possibility of reverting the hyperplastic gland to the colloid type by iodine.

Table 3 represents three animals in which an unrestricted diet of table scraps was coupled with a daily dose of two drops of syrup of iodide of iron. The results show a degree of hyperplasia, less in degree than in the corresponding controls, but nevertheless

TABLE 3

| NO. | SEX | INITIAL WEIGHT | CHANGE IN WEIGHT | THYROID GLAND | | | | DURATION OF EXPERIMENTS |
|-----|-----|----------------|------------------|---------------|------------|------------------|---------------------------|-------------------------|
| | | | | Gross weight | Net weight | Per cent colloid | Mgs. per kilo body weight | |
| 6 | M | 3780 | 770 | 0.570 | 0.438 | 22.99 | 115.7 | 3 weeks |
| 7 | M | 2250 | 1300 | 0.364 | 0.3435 | 5.86 | 152.6 | 3 weeks |
| 8 | F | 1080 | 1270 | 0.165 | 0.1070 | 17.99 | 99.4 | 3 weeks |

high. Like the preceding series the content of colloid is high, and the cells of all three glands contain small globules of colloid. Mitoses were abundant in all three glands, but most numerous in the glands from animal number seven in which the hyperplasia was highest, and the reaction to the iodides, as indicated by the content of colloid, lowest. In this gland mitoses were so abundant that often three or four might be seen in a single field of the 3 mm. apochromatic objective.

This series of experiments shows that, given the conditions which by themselves produce hyperplasia, iodides are not able *per se* to inhibit the hyperplasia, and that the hyperplasia is not due to deficiency of iodine. This result, at first sight contrary to Marine's conclusions, is not really so, since Marine has recognised that in the thyroid hyperplasia of the trout there are periods of greater and less susceptibility to iodine administration, and that the milder degrees react more promptly to iodine than the more severe ones. It may be presumed that mitosis and secretion are to a certain degree mutually exclusive, and that, therefore, the susceptibility of the gland to iodine is inversely proportional to the rate of hyperplasia. In the same way one might assume that, to the extent to which normal secretion could be maintained hyperplasia would be prevented, and thus iodine given at suitable times would prevent hyperplasia. In the present series, however, the impulse to hyperplasia is too strong for the iodine to overcome.

Table 4 represents the results in eight animals kept for a period of three weeks on a diet nearly sufficient to maintain constant weight. In one group, consisting of numbers 9, 13 and

TABLE 4

| NO. | SEX | INITIAL WEIGHT | CHANGE IN WEIGHT | THYROID GLAND | | | | DURATION OF EXPERIMENTS |
|-----|-----|----------------|------------------|---------------|------------|------------------|---------------------------|-------------------------|
| | | | | Gross weight | Net weight | Per cent colloid | Mgs. per kilo body weight | |
| 9 | M | 2660 | -300 | 0.148 | 0.118 | 20.18 | 44.0 | 3 weeks |
| 13 | F | 1020 | 15 | 0.098 | 0.094 | 4.04 | 92.1 | 3 weeks |
| 17 | F | 2660 | -150 | 0.180 | 0.137 | 3.99 | 50.44 | 3 weeks |
| 10 | M | 3000 | -80 | 0.170 | 0.1156 | 1.4 | 38.5 | 3 weeks |
| 14 | M | 1370 | -190 | 0.091 | 0.084 | 7.3 | 61.3 | 3 weeks |
| 15 | M | 3880 | -280 | 0.360 | 0.327 | 9.1 | 84.2 | 3 weeks |
| 11 | F | 2270 | 50 | 0.118 | 0.1105 | 6.36 | 48.6 | 3 weeks |
| 16 | M | 2430 | 70 | 0.093 | 0.0793 | 14.67 | 32.65 | 3 weeks |

17, the diet consisted of raw beef, free from fat and tendon, 5.4 g.; beef fat, 3 g.; and bread 7 g., per kilo of body weight. The second group, numbers 10, 14, 15, received daily, cheese, 3.6 g.; bread, 7 g.; and beef fat, 2.4 g., per kilo. Group three, including numbers 11 and 16, received daily, boiled egg, 9.4 g.; fat, 2.4 g.; bread, 7 g., per kilo.

Number 9, which shows a loss of 300 grams in weight, refused throughout to eat the bread. Numbers 13 and 14 each had a severe infection in a foot. Number 15 kept on the indicated diet for three weeks showed a gain in weight of 120 g. Then the right lobe was removed and the animal placed on the mixed unrestricted diet. It died on the fourth day after operation from infection in the wound, having lost meanwhile 400 g. in weight.

This series is remarkable for the low gross weights of the thyroid glands as well as for the low ratio of the corrected weight to body weight. Histologically, all the glands present the normal picture, though there is much variation, as indicated in the table, in the amount of colloid present. This variation, as well as the variation in the weight of the thyroid tissue per kilo of body weight, probably represents initial differences in the glands.

The series shows that the hyperplasia can be controlled by diet alone.

That the failure to show hyperplasia in this series is not due to a mild degree of starvation, as might be suggested by the fact that some of the animals lost weight, is indicated by cases 13 and

15, in which weight was gained, and by cases 21 and 22, in the next table, in which likewise weight was gained during the experiments, but in which, nevertheless, the thyroid gland remained small.

Table 5 represents the results of a series of experiments undertaken with a view of determining whether any particular element in the dietary was the cause of the hyperplasia. In this series, number 19 received twice, number 20 two and a half times, number 21 three times, number 22 four times, and number 23 five times the amount of meat indicated in the dietary for table 4. Number 20 refused bread one day of the twenty-one days that the feeding lasted, number 23 refused it four days. In all other cases the full ration provided was eaten.

TABLE 5

| NO. | SEX | INITIAL WEIGHT | CHANGE IN WEIGHT | THYROID GLAND | | | | RATIO INCREASE MEAT | DURATION OF EXPERIMENTS |
|-----|-----|----------------|------------------|---------------|------------|------------------|---------------------------|---------------------|-------------------------|
| | | | | Gross weight | Net weight | Per cent colloid | Mgs. per kilo body weight | | |
| 19 | F | 1960 | -40 | 0.142 | 0.1377 | 2.95 | 70.32 | 2.0 | 3 weeks |
| 20 | F | 2380 | -60 | 0.144 | 0.1398 | 2.69 | 58.72 | 2.5 | 3 weeks |
| 21 | F | 1350 | 70 | 0.074 | 0.063 | 14.36 | 46.94 | 3.0 | 3 weeks |
| 22 | F | 2490 | 140 | 0.156 | 0.131 | 16.06 | 52.54 | 4.0 | 3 weeks |
| 23 | M | 3500 | 450 | 0.455 | 0.451 | 0.77 | 129.00 | 5.0 | 3 weeks |

In four out of the five cases in this series the results as regards the size of the gland and the presence of mitoses were similar to those of table 4. In the fifth case, namely number 23, the animal which received the largest meat ration, the hyperplasia was of very high degree. Numbers 19 and 20 of this series were remarkable for the high content of secretion antecedent in the cells themselves. In 19 this substance filled the basal half of the cell. Numbers 21 and 22 had a smaller store of intracellular secretion but a relatively high content of intrafollicular colloid. Whether these differences were the result of the experimental conditions or mere accidental differences in the animals could not be determined from this one series, and the animals to repeat the experiment were unfortunately not available. The result in number 23

suggests that the hyperplasia is due to the protein fraction of the food, but further experiments are necessary to establish this fact.

These experiments show that the spontaneous hyperplasia observed in the thyroid gland of the opossum under laboratory conditions can be produced and controlled by diet alone, and that given the conditions dietary and otherwise which produce an active hyperplasia iodine will not inhibit the reaction. At all periods of the hyperplasia and to an extent which is proportional inversely to the rate of hyperplasia iodides will cause the storage of intrafollicular colloid. The experiments also emphasize the necessity in studies on the reactions of the thyroid gland, of controlling closely the conditions under which the animals are kept, and of reducing the gland as far as possible by diet and iodides to a normal base before the experiments are begun.



THE VASCULAR DRAINAGE OF THE ENDOLYMPHATIC SAC AND ITS TOPOGRAPHICAL RELATION TO THE TRANSVERSE SINUS IN THE HUMAN EMBRYO

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SIX FIGURES

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INTRODUCTION

In a previous paper (Streeter '14) dealing with some experimental studies on amphibian larvae, it was shown that in the tadpole the endolymphatic sac always lies in close apposition to the membranous roof of the hind-brain. This relation exists not only in normal specimens, but it was also found that in specimens where the ear vesicle had been rotated or transplanted by operative procedure, the endolymphatic sac in the subsequent self-correction of posture, succeeds in most cases in attaching itself to the membranous chorioidal roof in the normal manner.

This interesting topographical relation of the endolymphatic sac in the tadpole, induced the writer to examine more closely the endolymphatic sac in later human embryos, and it is the purpose of the present paper to outline the results of such a study in embryos from 20 mm. to 240 mm. crown-rump length.

It has long been known that in elasmobranchs the endolymphatic appendage opens directly on the surface of the body and that the surrounding sea-water can thereby pass directly through the endolymphatic duct to the cavities of the labyrinth. The arrangement that we have referred to as existing in the tadpole, suggests that we have there quite a different source of access for the endolymph. At any rate, it is evident that the contact existing between the endolymphatic sac and the membranous roof of the hind-brain affords favorable structural conditions for an interchange of substances between the cerebro-spinal fluid and the endolymph, either by diffusion or by a secretory activity of the separating epithelial membranes. The endolymphatic appendage also in the human embryo serves as an absorption-apparatus or one for regulating the endolymph, that is, if we may judge from its structural and topographical characteristics. The condition, however, in human embryos becomes somewhat more complicated than that in the tadpole in that here the sac is separated very early from the chorioidal membrane by the development of the dura mater and the intervening arachnoid-pial membrane. Instead of attaching itself to the membranous roof of the hind-brain, the sac projects against one of the large veins of the dura mater. Furthermore, it does not apply itself directly against the vein wall, but is separated from it by an intervening capillary plexus, which in turn drains into the vein. As far as the writer knows the character and connections of this endolymphatic capillary plexus is described here for the first time. As to its functional significance we must for the present limit ourselves to the above suggestion and in the following paper attention will be directed only to its morphology as seen in the typical stages of its development.

MATERIAL AND METHODS

The specimens which were examined microscopically in connection with this study consist of a group of human embryos, measuring from 21 mm. to 240 mm. (crown-rump) long, that is, from about the eighth to the twenty-eighth week of fetal life. They all belong to the Collection of the Department of Embry-

ology of the Carnegie Institution of Washington. The specimens in most cases had been injected with India ink through the umbilical vein and had been prepared in serial sections. In some cases after injection and fixation they were dissected so as to make total preparations which were rendered transparent in wintergreen oil and were examined under the binocular microscope. For purpose of topographical determinations, profile reconstructions were made of several of the embryos that had been cut serially and in some instances the structures were modelled after the Born wax-plate method. These will be specified under their separate descriptions. Although other embryos were examined the following list includes those that were chosen as best representing the stages of growth of the endolymphatic sac and its blood-vessels.

TABLE 1.

| EMBRYO NO. | CROWN-RUMP LENGTH | THICKNESS AND DIRECTION OF SECTION | VASCULAR INJECTION |
|------------|-------------------|------------------------------------|-------------------------------------|
| 460 | 21 mm. | 40 μ trans. | India ink. Wax-plate reconstruction |
| 632 | 24 mm. | 100 μ sagit. | India ink. Profile reconstruction |
| 449 | 34 mm. | 100 μ sagit. | India ink. Serial examination |
| 96 | 50 mm. | 100 μ sagit. | 0 Profile reconstruction |
| 448 | 52 mm. | 100 μ sagit. | India ink. Serial examination |
| 458 | 54 mm. | 0 | India ink. Cleared specimen |
| 1018 | 130 mm. | <i>Left side</i> | India ink. Profile reconstruction |
| | | 50 μ trans. | |
| | | <i>Right side</i> | |
| 1131 | 240 mm. | 0 | India ink. Cleared specimen |
| | | 100 μ trans. | 0 Serial examination |

HISTORICAL

In the opinion of the earlier embryologists the endolymphatic appendage represents the last portion of the ear vesicle that is attached to the skin, and which becomes drawn out into a stalk-like elongation as the vesicle recedes from the surface. They further pointed out that it corresponds to the narrow tube found in Selachians that passes dorsally through the cartilagenous skull to reach the surface of the head where it opens and thereby constitutes a canal that leads from the outside directly to the laby-

rinth. In this instance the ear vesicle remains attached to the skin throughout the whole period of its development. In other vertebrates it persists only as an embryological remnant of varying size that terminates as a blind sac under the dura mater and is apparently of no further use (Balfour '81, Hoffman '90, Hertwig '98).

This was the prevailing view regarding the endolymphatic appendage until results that conflicted with it were reported by Poli '97 and Netto '98. These investigators found that in reptiles and amphibians it is the lateral surface of the ear vesicle that is last to be detached from the skin, at a place clearly remote from the dorsal tip that gives origin to the endolymphatic duct. It was also found that in some cases the endolymphatic appendage does not make its appearance until after the detachment from the ectoderm is completed. Keibel '99 was strongly influenced by the condition existing in the embryo of the chick, where the separation of the otic vesicle from the ectoderm occurs relatively late and in fact the last point of attachment does occur at the dorsal tip of the endolymphatic appendage, and he therefore supported the original view of Balfour '81. He quite correctly defends the opinion that the tube in Selachians connecting the inner ear with the ectoderm is the same as the endolymphatic duct of the vertebrates. The conditions found in amphibians by Netto '98, where the endolymphatic duct does not develop until a considerable time after the complete detachment of the ear vesicle, he explains as a shifting in the time of occurrence of the ontogenetic as compared with the phylogenetic processes.

Subsequently the origin of the endolymphatic sac was carefully reviewed by Krause '01 who had an abundance of material for a comparative anatomical study. He showed that in reptiles the point of separation of the ear vesicle from the ectoderm has nothing to do with the dorsal pointed end of the vesicle from which the endolymphatic duct arises. While in birds, as described by Keibel '99 and others, it corresponds exactly to the tip of the endolymphatic duct. In mammals it also corresponds approximately to the tip of the endolymphatic duct, but here the

duct does not form until after or just at the completion of the detachment of the ear vesicle. In other words the separation point of the ear vesicle is a variable one and is not to be confused with the question of the homology of the endolymphatic duct. As regards the latter, Krause concludes that the endolymphatic duct of higher vertebrates is completely homologous with the canal that connects the labyrinth in Selachians with the surface of the head.

This, in brief, is the present status of our information regarding the endolymphatic duct in its general embryological aspects. As to its histology and blood supply we are primarily indebted to Boettcher '69. This investigator made razor-serial sections of the endolymphatic appendage of the adult cat and new-born babe. He, first of all, established the fact that it does not degenerate in mammals as was thought by contemporary investigators, but develops further and persists through life as an epithelial canal that connects with the two vestibular sacs, and forms an important part of the labyrinth. The terminal part spreads out (new-born babe) into a flattened sac 0.6 mm. wide, and is embedded in the connective tissue of the dura. This sac he describes as made up of cuboidal pavement epithelium, closely under which, and sometimes resting directly against it, are found capillary loops filled with red blood cells. The walls of the sac are somewhat irregular, due to the presence of small epithelial pockets which project outward into the periosteum or bone, and also papilla-like processes or folds which extend into the lumen of the sac. Both varieties are provided with capillary vessels. The capillaries are described as losing themselves in the periosteum. In another place he describes the small vessels of the vestibular aqueduct at its bony exit as uniting to form a common stem that empties into the inferior petrosal sinus. These significant observations of Boettcher have received scant attention from subsequent writers and do not seem to have resulted in further investigation of these interesting conditions.

Hasse '73 to whom we owe the generally accepted terms 'endolymphatic duct' and 'endolymphatic sac,' and who contributed many observations on the anatomy of the labyrinth,

speaks of the endolymphatic appendage as a tube extending from the labyrinth to the cranial cavity where it either ends blindly as an 'epicerebral lymph space' or opens into the general epicerebral lymph space (p. 768). Elsewhere (p. 792) he describes a small funnel-shape flaring process of the endolymphatic sac that penetrates through a small opening in the dura and there fuses with the arachnoid, thus establishing a communication between the 'cavum endolymphaticum' and the 'cavum epicerebrale.' The function of the endolymphatic appendage, according to Hasse, is threefold: 1, the sac, during embryonal life, is an epithelial secretory organ that furnishes the endolymph; 2, in the adult, it is either a closed sac that secures new materials for the endolymph by endosmosis from the epicerebral spaces, or it is an open sac through which the epicerebral fluid flows directly into the chambers of the labyrinth; 3, the endolymphatic sac is a reservoir for endolymph which serves as an expansion tank that relieves the pressure when it becomes too great in the labyrinth.

The investigators who have studied the blood supply of the labyrinth do not seem to have directed much attention to the vascularization of the endolymphatic appendage. They have done little more than to confirm the observation of Cotugno, made a century and one half ago, that a vein draining the vestibule and the canals accompanies the endolymphatic duct and empties into one of the dural sinuses. The most careful description is that of Siebenmann '94 who showed, as others had done for the aquaeductus cochleae, that the veins of the vestibular aqueduct (endolymphatic appendage) though originally accompanying the duct, become separated later in their own bony canal, which he designated as the 'canalis accessorius aquaeductus vestibuli.' Eichler '92 who studied the blood-vessels of the human labyrinth confined his attention to the cochlea.

Shambaugh '03 describes the endolymphatic duct as incased by capillaries which are supplied by an arteriole coming usually from the posterior vestibular artery, and are drained by a vein that empties into the transverse vestibular vein. Where the endolymphatic sac was preserved it was found to be drained by a small dural vein.

ENDOLYMPHATIC APPENDAGE DURING FIRST TWO MONTHS

The features with which we are chiefly concerned in the present paper do not become established until toward the end of the second month (embryos over 30 mm. long). A review, however, will be briefly made of the form and relations of the endolymphatic appendage prior to that time. For a more detailed description

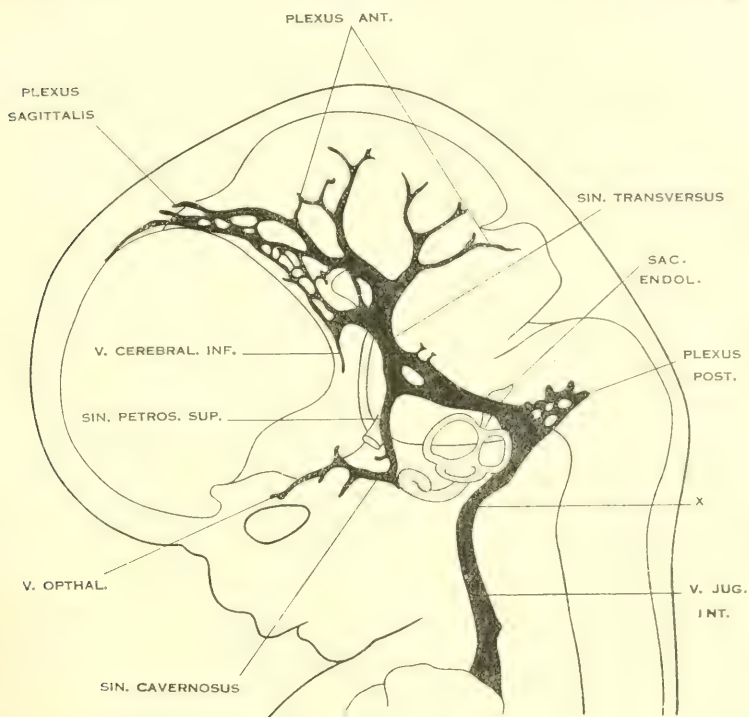


Fig. 1 Profile reconstruction showing the topography of the membranous labyrinth and the endolymphatic appendage in a human embryo 24 mm. long (No. 632, Carnegie Collection). The principal head veins are shown in solid black. Enlarged about 4 diameters.

with illustrations the reader is referred to a paper previously published on the development of the membranous labyrinth (Streeter '06) and to a recent paper on the dural sinuses in which special attention is given to the topography of the labyrinth at its different stages (Streeter '15).

In embryos 4 mm. long the ear vesicle consists of a simple slightly elongated spherical sac that lies in the space between the primary head vein and the lateral wall of the hind-brain. At its dorsal end can be recognized a rounded pouch-like projection which is quite distinctly marked off from the rest of the vesicle. This is the early endolymphatic appendage. It is in relation both with the brain wall and the skin, but is separated from them by a scant amount of mesenchyme, in which can be seen minute blood-vessels that communicate with the middle and posterior dural plexuses. The appendage points toward the rhombic lip, but does not quite reach its dorsal margin.

In its subsequent growth the endolymphatic appendage rapidly becomes more clearly differentiated from the remainder of the labyrinth. It takes on a slender tubular form, whereas the vestibular part of the labyrinth expends into a voluminous triangular pouch. The tubular character of the endolymphatic appendage is pronounced in embryos from 9 mm. to 14 mm. long. By its elongation it passes over the rhombic lip and in 14 mm. embryos we find the tip of it overlapping the ventrolateral part of the thin chorioidal roof of the fourth ventricle. It, however, does not lie in direct contact with this membrane as is the case in tadpole larvae, but is always separated by a thin layer of the surrounding mesenchyme.

At about the time of the closing-off of the semicircular canals (embryos 15 mm. long) the simple tubular form of the endolymphatic appendage is gradually modified by the expansion of its distal half into a flattened fusiform sac, which from then on is recognized as the endolymphatic sac as distinguished from the remaining proximal part, the endolymphatic duct, that connects it with the rest of the labyrinth. The endolymphatic sac lies lateral and caudal to that part of the chorioidal membrane that is to form the lateral recess of the fourth ventricle. It lies close against it, but is always separated from it by the tissue that is to form the arachnoid and dural membranes.

Simultaneously with the formation of the semicircular canals and the differentiation of the endolymphatic sac there occurs an alteration in the large dural veins in this neighborhood that plays

an important part in its topography. This consists in the replacement of the primary head vein by a more dorsally situated longitudinal channel. The middle dural plexus instead of draining, as

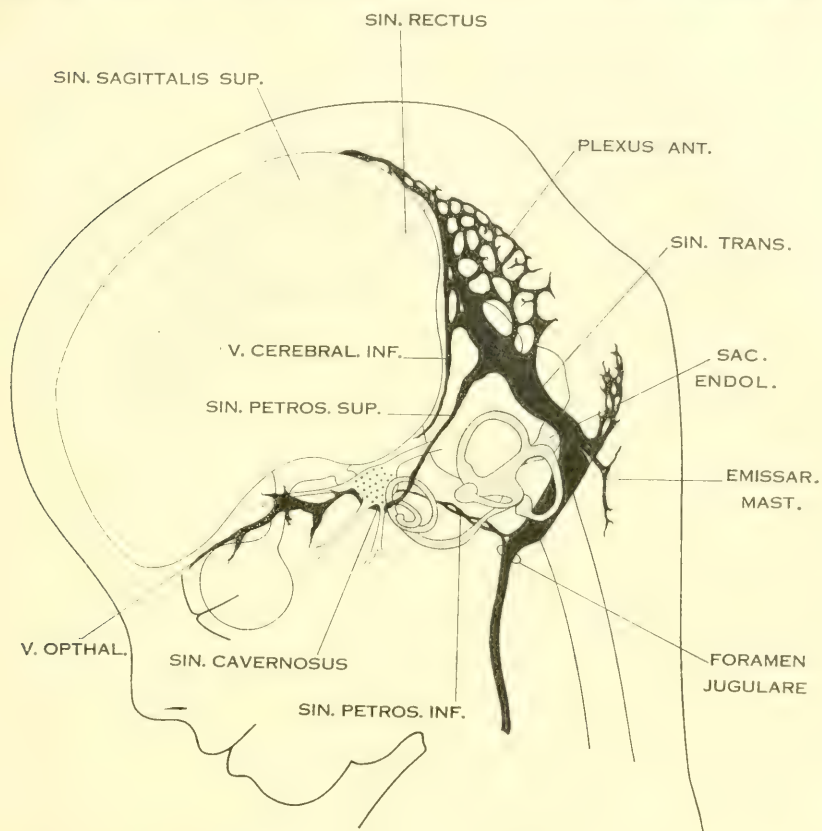


Fig. 2 Profile reconstruction showing the topography of the membranous labyrinth and endolymphatic appendage in a human fetus 50 mm. long (No. 96, Carnegie Collection). The endolymphatic sac is partly covered by the transverse sinus, which with the other head veins is shown in solid black. Enlarged about 4 diameters.

formerly, into the primary head vein drains caudalward into the posterior dural plexus. Soon afterward the anterior dural plexus, in a similar manner, changes its direction of drainage and instead of continuing to drain into the cephalic end of the primary head

vein, it unites with the middle dural plexus and they both drain into the posterior dural plexus and through it into the internal jugular vein. Due to these alterations in the drainage of the anterior and middle dural plexuses the greater part of the primary head vein disappears and we find it replaced by the more dorsally situated channel that is to become the transverse sinus. This channel forms in a groove in the dorsal margin of the otic capsule. Topographically it passes longitudinally in the space between the two vertical canals and the endolymphatic sac. The general relation of these structures is shown in figures 1 and 2 which are reproduced from the paper previously referred to (Streeter '15). The canals are separated from the sinus by their cartilagenous envelope. The endolymphatic sac, however, like the transverse sinus itself does not become encased by cartilage and lies against the median wall of the latter, separated from it only by a small amount of loose embryonic connective tissue in which both are embedded. This close relation which becomes established between the endolymphatic sac and the transverse sinus in 18 mm. embryos, continues as a permanent condition. At first (fig. 1) when the endolymphatic sac has a vertical position, it completely overlaps the median surface of the sinus. Subsequently as the cranium enlarges, this part of its wall is crowded outward and downward into a more horizontal position and partakes in the formation of the floor of the posterior cerebral fossa. We then find the endolymphatic sac resting on the dorsal surface of the sinus and furthermore the sinus becomes relatively larger than the sac and is then only partly overlapped by the latter.

Though closely related to the chorioidal membrane of the lateral recess, the endolymphatic sac becomes more and more clearly separated from it as the dural and arachnoidal tissues become differentiated. On the other hand, though resting against the transverse sinus, there is a scant amount of loose embryonic connective tissue separating the two. Running through the meshes of this connective tissue can be seen blood capillaries that form a plexus which empties into the transverse sinus. This plexus anastomoses with the vessels of the labyrinth by com-

munications along the endolymphatic appendage. It also anastomoses with the posterior dural plexus. These blood-vessels and their communications can be recognized in embryos 20 mm.

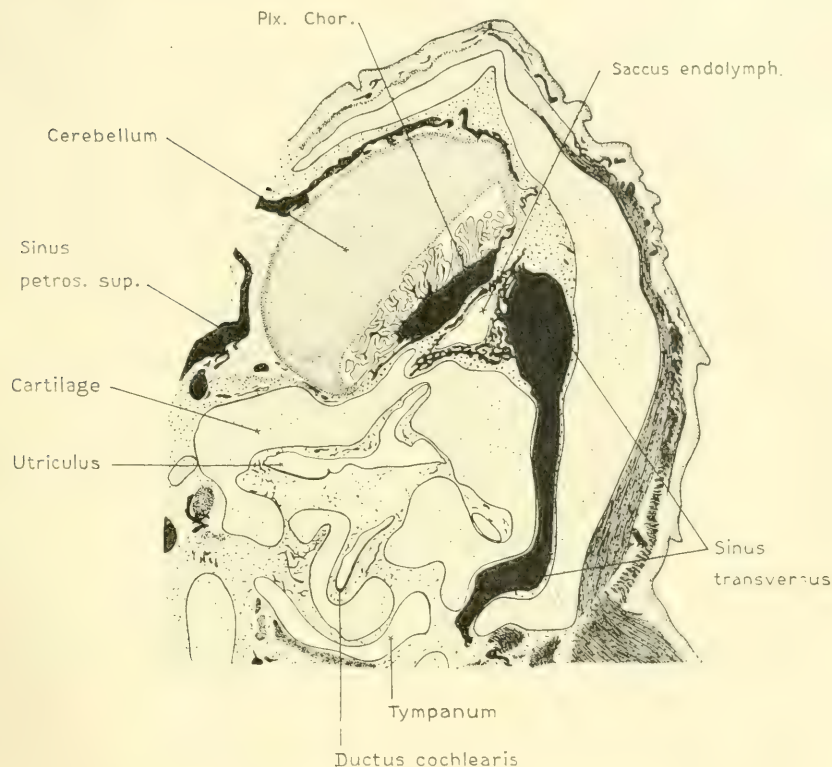


Fig. 3 Sagittal section through the ear region of a human fetus 52 mm. long (No. 448, Carnegie Collection). The blood vessels are injected with India ink and are represented in solid black. The endolymphatic sac is outlined as a clear space and surrounding it can be seen its dense capillary plexus and the manner in which this drains into the transverse sinus. Enlarged about 10 diameters.

long, but subsequent to that they rapidly increase in size and importance, and in embryos 50 mm. long obtain a characteristic appearance which we shall now proceed to describe.

ENDOLYMPHATIC APPENDAGE DURING THE THIRD MONTH

The topography and vascular drainage of the endolymphatic sac in embryos about 50 mm. (crown-rump) long are shown in figures 2, 3 and 4. In figure 2 can be seen the general posture of the labyrinth and the relation of its component parts to the dural sinuses. This figure is drawn from a profile reconstruction of the labyrinth, dural veins and central nervous system in an embryo 50 mm. long (No. 96, Carnegie Collection). The reconstruction was prepared by projecting the serial sections on transparent papers which were then superimposed and all traced on one sheet. It will be noted that the endolymphatic sac passes upward so that its dorsal one-third rests against the median surface of the transverse sinus, opposite the chorioidal roof of the ventricle of the hind-brain. It does not project above the sinus as in the younger stage shown in figure 1.

A section through this region is shown in the accompanying figure 3. This is a portion of a sagittal section through a human embryo 52 mm. long (No. 448, Carnegie Collection). Before the embryo was prepared in serial sections its vascular system was injected with India ink through the umbilical vein. This injection mass is shown in the drawing in solid black. The section passes antero-posteriorly through the lateral part of the cerebellum, and includes a portion of the ventricle with the chorioidal villi projecting into it. At the base of the villi there is a collection of the injection mass which apparently is an extravasation. This is separated from the endolymphatic sac and its vessels by the dura which is already fairly well outlined, though it is not represented in the drawing.

The feature to which particular attention should be given is the capillary plexus surrounding the endolymphatic sac. Its general character is indicated, and it can also be seen that it drains by several outlets into the transverse sinus. On following it through the sections of the series it is found that it completely envelops the endolymphatic sac and duct. It can be traced centrally within the cartilage as a finely meshed tubular covering of the duct extending to the region where the duct arises from the

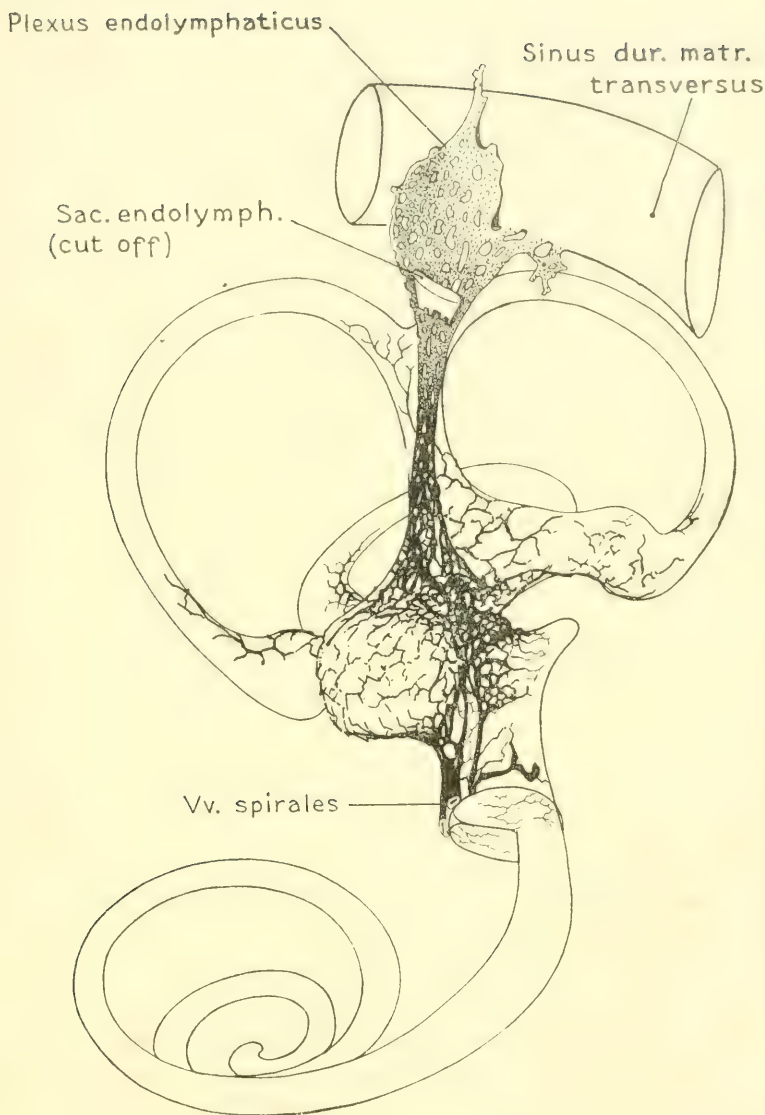


Fig. 4 Camera lucida drawing showing the endolymphatic plexus and its communications in a human fetus 54 mm. long (No. 458, Carnegie Collection). The blood vessels were injected with India ink and the whole rendered transparent with wintergreen oil. A portion of the plexus was removed to show the contained endolymphatic sac, and part of the sac was also removed in order to show the drainage of the plexus. The vessels of the rest of the labyrinth are only filled in far enough to show their communication with the endolymphatic plexus. Enlarged about 17 diameters.

utricle and saccule. At this point it anastomoses with the vessels of the vestibular part of the labyrinth. The vessels belonging to the vestibule and canals are more sparse; a portion of the cochlea, however, seems equally as well provided as the endolymphatic appendage. It is to be remembered that we are dealing with an injected embryo and the meshes of this plexus are doubtless distended, so that the picture we obtain shows them more prominently than would be the case in uninjected material. The topography and communications of the endolymphatic blood plexus are shown more completely in figure 4. This is an outline drawing of the labyrinth and its blood-vessels in a human embryo 54 mm. long (No. 458, Carnegie Collection). The blood-vessels were injected with India ink and after fixation the head of the embryo was dissected and the desired portions of it were dehydrated and cleared in wintergreen oil. Figure 4 shows the right labyrinth as seen in such a specimen. The injected vessels in the region of the vestibulo-cochlear junction are shown in solid black and also their continuation into the endolymphatic plexus inclosing the endolymphatic duct. The continuation of the plexus toward the lateral sinus is shown in stipple. In the region of the endolymphatic sac a part of the plexus is represented as cut away. The greater part of the sac is also cut away in order to expose more completely the outer leaf of the plexus, that intervenes between the endolymphatic sac and the sinus, and its characteristic communications with the sinus. The sac is quite flat and when it is intact it corresponds in contour to that portion of the plexus that has been left. The reader will be able to form a picture of the whole apparatus by imagining the rest of the sac back in place and covered in by the inner leaf of the plexus.

From an examination of figures 2, 3 and 4, we see, therefore, that in embryos about 50 mm. long the endolymphatic appendage consists of a narrow duct that widens out into a broad flattened sac that lies between the chorioidal membrane of the lateral recess and the transverse sinus. It is separated from the former by the dura and is separated from the latter by the endolymphatic plexus. This plexus consists of thin walled capillaries

which everywhere inclose the duct and sac. In the distended state, as in injected specimens, they virtually constitute a surrounding sheet of blood inclosed in endothelium, since the openings in the mesh are, as a rule, narrower than the blood channels themselves. There is some tendency at this time, and it becomes more marked later on, to the formation of principal channels in this plexus. The plexus anastomoses centrally with the other blood vessels of the labyrinth. Distally it drains by several openings into the transverse sinus. In addition it anastomoses with a coarser plexus of veins that lies between the dura and the cartilaginous skull in the neighborhood of the sinus. In this same region there are some small arteries of the dura mater that seem to communicate by minute branches with the endolymphatic plexus. There were very few of these and their arterial nature could not be determined with certainty.

ENDOLYMPHATIC APPENDAGE AT END OF FOURTH MONTH

The endolymphatic plexus gradually changes its character as we advance to older fetuses. Instead of a fairly uniform meshwork that envelops evenly all parts of the appendage, part of it takes the form of larger and simpler channels that become more or less separated from the remainder of the plexus while the latter continues as a fine meshwork closely applied to the surface of the appendage. The finer plexus drains into the larger channels which in turn drain into the transverse sinus.

In order to determine the topography and vascularization of the endolymphatic appendage at this period, a well hardened fetus, 130 mm. crown-rump length, was selected in which the blood-vessels had been injected through the umbilical vein with India ink (No. 1018, Carnegie Collection). The part of the skull on each side containing the labyrinth was removed, care being taken to preserve the dura. The specimen from the right side was dehydrated and cleared in wintergreen oil and studied as a transparent specimen. The left one was decalcified and cut in serial sections and a profile reconstruction was made of the labyrinth and larger vessels. By combining the reconstruction with the study of the transparent specimen it was possible to

ascertain very definitely the relations of the structure with which we are concerned.

A camera lucida drawing of the endolymphatic plexus and its connecting vessels is shown in figure 5, as they are seen in the cleared specimen mentioned above. In the same drawing is introduced a profile reconstruction of the endolymphatic appendage prepared from serial sections of the other labyrinth. From an examination of this figure it will be seen that the endolymphatic appendage is divisible into a duct and a sac. The duct is further divisible into a proximal flaring portion and a narrow portion that connects this with the sac. It can be seen in sections that the proximal flaring portion possesses thin walls that show a tendency to be thrown in folds. The endolymphatic sac consists of a flattened blind pouch with a rounded contour. Microscopic examination shows that its walls consist of a single layer of cuboidal epithelium which is uniform throughout the sac except at its distal extremity where it narrows into a tubular process whose epithelium retains the embryonic character. In its general topography the endolymphatic sac maintains its former relations and its distal part is found overlapping the dorso-medial wall of the transverse sinus.

On examining the endolymphatic plexus in figure 5 it will be seen that it has undergone certain changes as compared with the younger stage shown in figure 4. A vascular plexus still envelops the appendage everywhere. This consists of a thin walled endothelial network whose meshes vary in size and pattern and lie closely against the epithelial wall of the appendage. In the drawing only the more prominent loops are shown; besides these there are everywhere small anastomosing capillaries that intervene between them. The network as a whole is richer over the sac and over the proximal flaring portion of the duct and is more scant over the narrow portion of the duct. Running through the plexus there are a few larger channels that have been separated out. These form main drainage channels that become partially detached from the general plexus, though the latter continues to anastomose with them at frequent intervals. One of these is the so-called 'vena aquaeductus vestibuli.' This forms

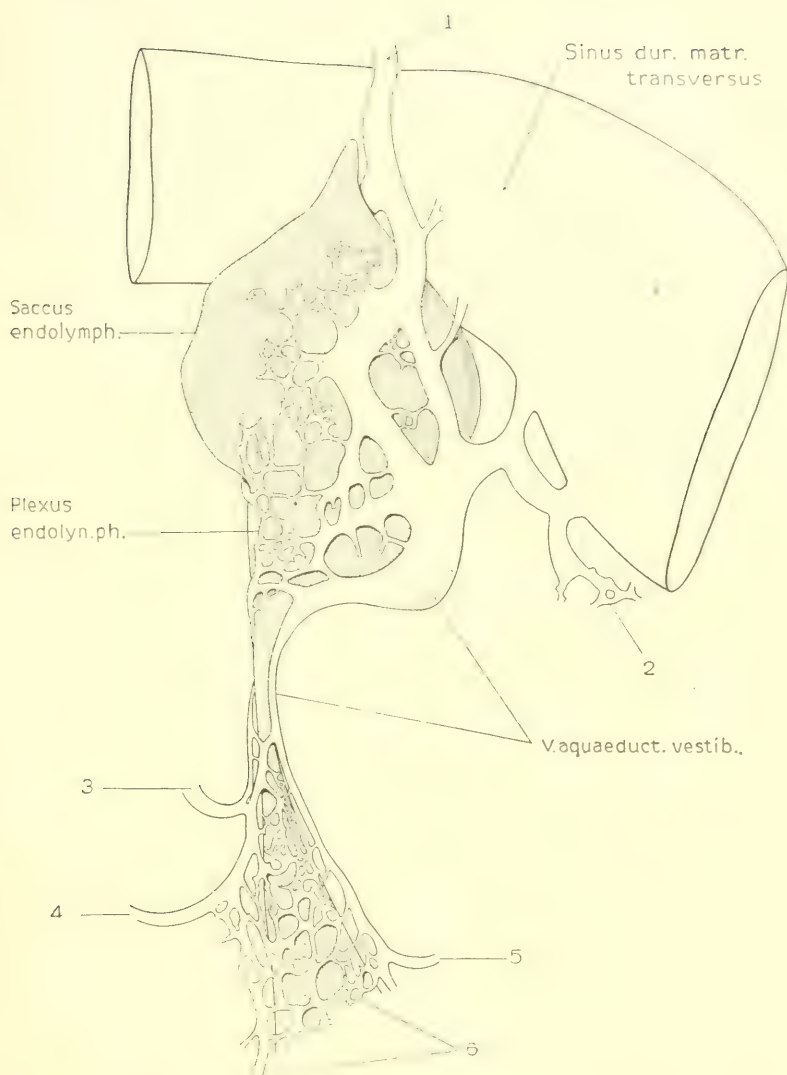


Fig. 5 Profile reconstruction of the endolymphatic appendage in a human fetus of 130 mm. crown-rump length (No. 1018, Carnegie Collection). Combined with it is a camera lucida drawing of the endolymphatic plexus, with its connections, made from the other labyrinth of the same specimen which had been cleared in oil. The numerals indicate communications of the endolymphatic plexus with other veins: 1 and 2, dural veins; 3, vein draining plexus on dorsal surface of utricle; 4, from plexus on median surface of utricle; 5, from posterior ampulla and adjacent part of utricle and saccule; 6, veins from median surface of saccule and cochlea. Enlarged $17\frac{1}{2}$ diameters.

along the borders of the endolymphatic duct. It may be regarded as having a group of tributaries from the remainder of the labyrinth. These are numerically indicated in figure 5 as follows: '3' is a vein draining the dorsal surface of the utricle from where it curves around at the base of the crus commune to join the endolymphatic system; '4' drains the plexus belonging to the medial wall of the utricle; '5' drains the plexus of the posterior ampulla and the adjacent posterior surfaces of the utricle and saccule; '6' indicates a group of anastomosing vessels from the median wall of the saccule through which it also communicates with the cochlear system. Opposite the narrow part of the endolymphatic duct these various channels are assembled into two vessels of which the one along the posterior margin of the duct is the principal one, and the one that persists as the v. aquaeductus vestibuli. Tracing it upward we find it receiving large tributaries from the plexus of the endolymphatic sac and at the same time enlarging into a wide channel along the caudal margin of the sac. In addition to the tributaries from the endolymphatic plexus it receives several tributaries from the plexus underlying the surrounding dura, such as '1' in figure 5. It empties into the transverse sinus by one or two openings in conjunction with adjacent dural veins.

In describing this plexus and the vena aquaeductus vestibuli it is simpler to think of the blood stream as flowing all in one direction, that is, toward the transverse sinus. In reality it is quite possible that, due to mechanical conditions, the plexus of the proximal part of the duct drains backward into the vessels of the rest of the labyrinth and in common with them through the veins of the cochlear aquaeduct. The natural drainage of the sac, however, is toward the transverse sinus. Under these conditions the narrow part of the duct is a 'divide' from which the blood flows in both directions, and through the same v. aquaeductus vestibuli.

ENDOLYMPHATIC APPENDAGE IN EMBRYOS DURING SEVENTH MONTH

To represent late fetal conditions of the endolymphatic sac, a fetus was selected weighing 948 gms., in formalin, and measuring 240 mm. crown-rump length (No. 1131, Carnegie Collection). The head of the fetus was removed and divided in bilateral halves. On one side a dissection was made exposing the endolymphatic sac which was done by carefully reflecting the dura. The form of the sac and its relation to the transverse sinus was found to be essentially the same as that shown in figure 5, so a drawing of it will not be repeated. On the other side of the specimen the dura was raised in one mass together with all the soft tissues between it and the bone; this included the endolymphatic sac, the periosteal vessels and part of the terminal portion of the transverse sinus. This was then embedded and prepared in serial sections, in a plane longitudinal to the duct and transverse to the sac. A simplified drawing of one of these sections is shown in figure 6.

In the drawing the endolymphatic sac is shown in heavy black stipple. It consists of a flattened sac embedded in the connective tissue that forms the substratum of the dura. Its distal portion overlaps the dorso-median surface of the sinus as in the previous stage. One new feature is found that was not present in the younger stages and that is that the epithelial wall of the sac projects irregularly in small longitudinal folds apparently thereby offering greater surface area. A characteristic fold of this kind is cut through in the section shown in figure 6. Such a fold gives the appearance of a double sac but tracing it through the sections shows that it is only an out-pocket whose lumen communicates with that of the main sac.

The dura mater merges gradually into a somewhat loose substratum of connective tissue that attaches it to the bony skull. This is schematically represented in the drawing and the raggedness of the bony surface of the dura is due to the difficulty in detaching the dura from the bone and also in part to the irregularity of the bone. In the meshes of the connective tissue of the

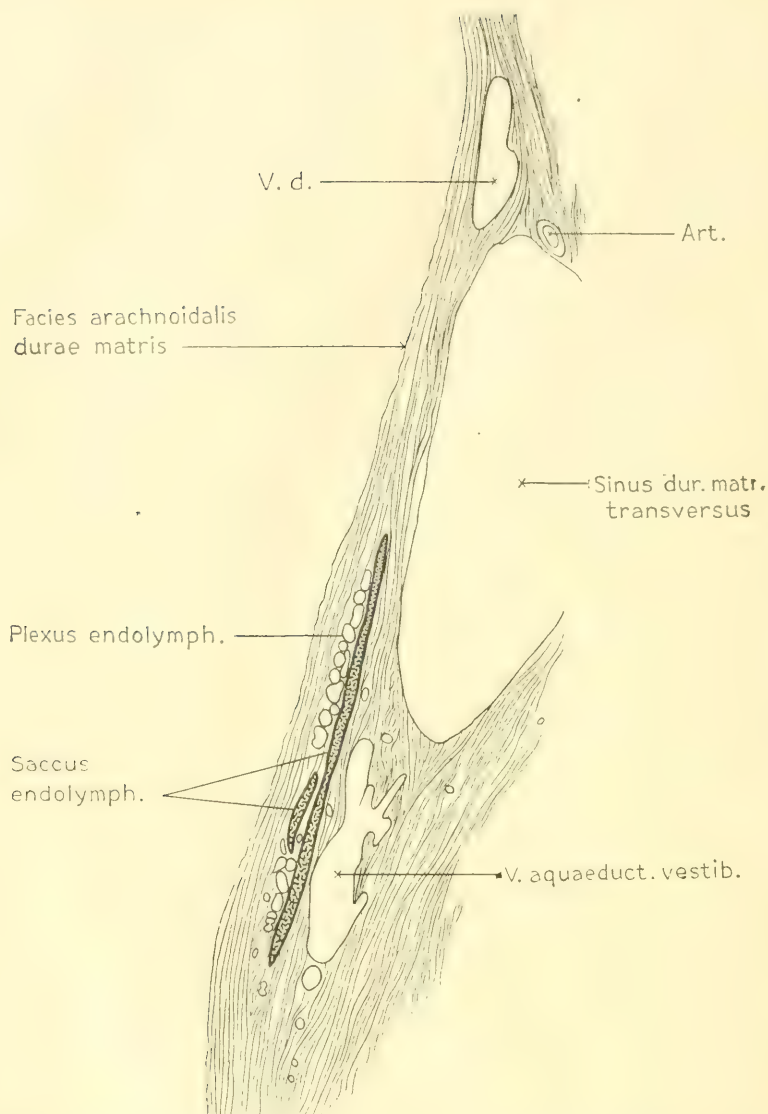


Fig. 6 Section through the endolymphatic sac showing its relation to the dura and blood vessels in a human fetus measuring 240 mm. crown-rump length (No. 1131, Carnegie Collection). Endolymphatic sac is stippled dark. Blood vessels are shown in plain white. The endolymphatic plexus is more dense on the median or upper surface of the sac; on the lateral or lower surface the plexus is partly replaced by the main channel through which it drains into the transverse sinus. 'V. d.,' a large dural vein; 'Art.,' artery. The arachnoidal surface of the dura is intact, but the bony surface was torn in the removal of the specimen from the bone. Enlarged 15 diameters.

dura are found numerous blood vessels which are shown in the drawing as white spaces. The largest of these is transverse sinus. A portion of its wall is missing having been injured in the removal of the dura from the bone. Around the endolymphatic sac is a thick plexus of thin walled veins which apparently is the same as the endolymphatic plexus which we have studied in the younger specimens. At the caudo-lateral surface of the sac they open into a large channel which in turn drains into the transverse sinus. This is the channel that follows along the endolymphatic duct and is known as the vena aquaeductus vestibuli. Other dural veins anastomose with it, but its primary communication is with the venous plexus of the endolymphatic sac. As this specimen did not include the intraosseus portion of the endolymphatic appendage the proximal connections of these veins could not be studied.

SUMMARY

From the above study of the endolymphatic appendage in human embryos the principal features in its development, topography and vascularization may be summarized as follows:

The endolymphatic appendage makes its appearance at the dorsal tip of the otic vesicle in embryos about 4 mm. long, whereupon it rapidly enlarges, forming an elongated tube that extends upward toward the chorioidal roof of the hind-brain. As it does this it becomes differentiated into two subdivisions: the distal half spreads out forming a broad flattened blind pouch, the *saccus endolymphaticus*; the proximal half, the *ductus endolymphaticus*, forms an elongated narrow tube connecting the distal part with the remainder of the labyrinth. The main features in this differentiation are completed in embryos 30 mm. long and at the same time the topographical relations of the appendage have assumed practically the adult conditions.

A prominent factor in the topography of the endolymphatic sac is its relation to the transverse sinus. The characteristic flattened form of the sac and the establishment of the sinus are to be seen at about the same time. From then on the sac always lies with its flat surface applied against the median wall of the sinus.

or the dorso-median wall as the base of the skull becomes more flattened out. The sac does not become incorporated with the rest of the labyrinth in the cartilaginous capsule, but like the sinus lies exposed in the floor of the posterior cerebral fossa and is covered in only by the dura mater.

Throughout the greater part of foetal life the endolymphatic appendage is ensheathed by a vascular plexus, the plexus endolymphaticus, which anastomoses on the one hand with the vessels of the rest of the labyrinth and on the other hand with the transverse sinus into which it drains through several openings.

This plexus makes its appearance at about the time of the differentiation of the appendage into its adult subdivisions of duct and sac. It can be plainly recognized in embryos 30 mm. long. In embryos 50 mm. long, it is well developed and at that time it forms a closely meshed web completely investing the appendage, whereby the latter is virtually inclosed in a sheet of blood from which it is separated only by the endothelium of the blood spaces.

In the course of its further enlargement and development in embryos 100 mm. long and over, the endolymphatic plexus becomes resolved into a few principal channels connected with which there remain parts of the original plexus. The plexus persists notably in the neighborhood of the endolymphatic sac.

One of the most constant channels that are developed through the endolymphatic plexus is the one forming the so-called vena aquaeductus vestibuli. This forms along the side of the endolymphatic duct and the posterior margin of the endolymphatic sac, and it constitutes a direct communication between the vascular plexus surrounding the labyrinth on the one hand, and the transverse sinus on the other. It may be a single or multiple channel. Through it is drained the plexus of the endolymphatic sac and also some of the dural veins of the immediate neighborhood.

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PROBLEMS OF HUMAN DENTITION

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TWENTY-EIGHT FIGURES

INTRODUCTION

During the last few years I have been occupied with anatomical and embryological researches upon the dentition of mammals and reptiles. The memoirs dealing with the results of these investigations are published in the Dutch or German language. My researches on the mammalian dentition were principally executed on human and other primate material. In consequence of these investigations, I have arrived at conclusions regarding some fundamental odontological problems, differing somewhat strongly from those generally accepted. However, as my conclusions are founded partially on the examination of a large amount of material and partially on the observation of heretofore unknown facts and relations, I believe that my points of view in some respects throw new light upon odontological problems.

The present paper discusses only some purely morphological problems of the primate dentition, especially with reference to the dentition of man. In the next essay I hope to treat of some embryological phenomena.

I have demonstrated in my "Odontologische Studien"¹ that the ontogenesis of the mammalian teeth shows peculiarities previously undescribed, the knowledge of which makes their developmental history in some degree different from the generally

¹ Odontologische Studien I. Die Ontogenie der Primatenzähne. Jena, Gustav Fischer, 1913. Odontologische Studien II. Die Morphogenie der Primatenzähne. Jena, Gustav Fischer, 1914.

accepted scheme. The embryological development of mammalian teeth is a process more complicated than the description of it as given in text-books of anatomy or odontology would lead one to suppose.

First I shall treat of the manner in which the dentition of man (and of all other platyrrhine Primates) is a development of the more primitive form of platyrrhine dentition. Then the problem will be discussed as to which set of teeth our molars belong (to the first or milk dentition, or to the second or permanent set of teeth); and finally I shall give my conception as to the future changes which will occur in human dentition.

FIRST PROBLEM: THE RELATION BETWEEN THE DENTITION OF
PLATYRRHINE AND CATARRHINE PRIMATES

As is generally known, one of the most striking anatomical differences between the two groups of Primates (the Platyrrhinae and the Catarrhinae) is that the monkeys of the New World possess three premolars and three milk molars in each jaw, whereas in catarrhine monkeys and likewise in man, there are only two of each of these teeth. There is some difference between the two families of American monkeys, the Hapalidae possessing two molars only, whereas in the Cebidae there are three of these teeth, as in all other Primates. Therefore the majority of New World monkeys have a set of teeth whose post-canine portion possesses one tooth more than the corresponding portion of the Old World monkeys, or of man.

It is a common view of anatomists and zoologists that the dentition of the latter evolved from that of the former group by the loss of one of the premolars. But there is no agreement as to the premolar which was reduced. Most investigators maintain that the first premolar of the platyrrhine monkeys is wanting in the catarrhine group. Therefore the first premolar of the latter should be considered the homologue of the second premolar of the former. Other investigators, on the contrary, assert that it is the third premolar of the Platyrrhinae which is wanting in the other families of the Primates. I do not agree with either of these views. My opinion as to the relation between the den-

tition of the Platyrrhinae and the Catarrhinae is wholly different. But there is one point common to both hypotheses worthy of special reference because it constitutes a weak side of each of the hypotheses.

The dental formula of the platyrrhine monkeys (save the Hapalidae) runs as follows:

$$\begin{array}{c} i_1, i_2, c, m_1, m_2, m_3. \\ I_1, I_2, C, P_1, P_2, P_3, M_1, M_2, M_3. \end{array}$$

and that of the catarrhine Primates:

$$\begin{array}{c} i_1, i_2, c, m_1, m_2. \\ I_1, I_2, C, P_1, P_2, M_1, M_2, M_3. \end{array}$$

In these formulas the elements of the milk dentition are written in small print and those of the permanent set of teeth in capital letters. The formulas as above written are not intended to give expression to any homology between the teeth of the two groups of primates. In accepting either of the hypotheses mentioned above one must keep in view the fact that the catarrhine dentition not only arose from the reduction and final loss of a premolar in the permanent set of teeth, but also of its predecessor in the milk dentition. It seems to me to be very important that in none of the recent genera of the New World monkeys is a reduction of a tooth to be seen either of the first or third milk molar, or of the first or third premolar. It is altogether probable that in the extinct ancestors of the catarrhine Primates such a reduction really took place. But one looks in vain both in the upper and lower jaw of this group of Primates for any proof that a tooth in the premolar region has been lost. In its whole extent this portion of the dental arch is always regularly constructed, the teeth standing very closely approximated. Furthermore in catarrhine monkeys a diastema, especially between the canine and first premolar is wanting. These facts surely are not very favorable to the opinion that the reduction in the number of the premolars in Primates happened in the common way—in consequence of the loss of a premolar and its predecessor in the milk dentition. For, in case such a process really happened, the situation of the lost tooth would be indicated by a diastema. The objection to

the usually accepted hypothesis is further strengthened by the consideration that the diminution from the primitive number of four premolars to three, which happened in the eocene Primates, can be followed step by step in the different well-known genera of their group of the common ancestors of all recent Primates.

The following objection may also be advanced. If really the first post-canine tooth in both dentitions should be reduced and lost, there is very strong ground to expect that in the embryological evolution of the dentition of man, apes or catarrhine monkeys, the anlage of this tooth or occasionally the tooth itself should be found in a rudimentary form and size. Without doubt the teeth are reduced and lost during the last phase of development in all mammals. Although the development of the human dentition in human embryos has been examined by a great number of investigators, there has never been found a single vestige of the anlage of a rudimentary milk molar immediately behind the canine.

I shall not consider in a detailed manner the current hypotheses, being of a wholly different opinion with regard to the relation between the dentition of American monkeys and that of Old World monkeys. This opinion may be briefly expressed as follows: The dentition of the catarrhine Primates (including man) with its two premolars is derived from an ancestral form with three premolars in two phases. The first phase was characterized by the reduction and final loss of the third molar of that ancestor. By this process a form resulted with three premolars and only two molars, just as we actually find in the group of the recent Hapalidae. The second phase was of an entirely different nature: during the same, the third milk molar developed into a permanent tooth, whilst the development of its successor (the third premolar) was suppressed, in consequence of which the number of permanent molars increased to three, as in the primitive form.

By this hypothesis the Hapalidae, with regard to their dentition, are placed on a higher level in the phylogenetical system than they occupy in the common systems of Primates. One is accustomed to consider the Marmosets the most primitive recent

representatives of the primate stem. It was never very clear to me upon which points in their anatomical structure this opinion is grounded. It is true that their nails, except those on the hinder thumbs, are formed like claws. But this peculiarity is a phenomenon of less value with regard to the problems of phylogenetical evolution than the indications supplied by the structure of the dentition. And in comparing the anatomy of the molars of the other platyrrhine monkeys—the Cebidae—with those of the Hapalidae, it becomes clear that nearly all Cebidae show a tendency to attain a developmental stage already accomplished by the Hapalidae, or the total reduction of the hindmost molar. In most genera of the New World monkeys—Cebus, Ateles, Chrysothrix, Pithecia, Nyctipithecus—the third molar is already reduced in a very large degree, having only a single root and a very small crown without cusp-differentiation. Usually this hindmost tooth of the Cebidae is a far more reduced element of the dentition than the third molar in man. The fact that the Cebidae approach a structure of their dentition already acquired by the Hapalidae, is to me a sufficient ground to place the latter on a higher level of phylogenetical evolution than the former. It is worthy of mention, that the investigation of Weber showed the brain of the Marmoset, although a lissencephalous one, to be relatively heavier than even that of man.

Therefore, I consider the dentition of the Hapalidae an intermediate form between that of Cebidae and catarrhine Primates, notwithstanding the reduced number of their molars. The loss of the hindmost molar, was the first step which led the platyrrhine ancestor of man to the more progressive dental structure peculiar to all Old World Primates. We will return later on to the cause of this reduction.

The primitive number of three molars was regained in consequence of the third milk molar becoming permanent and of the suppression of the development of the third premolar. This is, I admit, somewhat unusual in the evolution of dentition. But there are other examples, well-known to us, in which the same phenomenon took place, and in consequence of which the functional set of teeth became a mixed one, composed partially of

milk teeth and partially of teeth of the second dentition. The best known case is that of the Erinaceus. According to the very exact and ample researches of Leche, the functional set of teeth of this Insectivora is composed of elements of both dentitions. The above point of view therefore does not introduce a wholly new principle in odontology.

Before developing the different arguments upon which my hypothesis is based, I wish to summarize its essentials by means of some dental formulas. In these the symbols of the milk dentition are printed in small letters, the permanent teeth in capitals.

Dental formula of the Cebidae:

$$\begin{array}{c} i_1. i_2. c. m_1. m_2. m_3. \\ I_1. I_2. C. P_1. P_2. P_3. M_1. M_2. M_3. \end{array}$$

Dental formula of the Hapalidae:

$$\begin{array}{c} i_1. i_2. c. m_1. m_2. m_3. \\ I_1. I_2. C. P_1. P_2. P_3. M_1. M_2. [M_3] \end{array}$$

Dental formula of the Catarrhinae:

$$\begin{array}{c} i_1. i_2. c. m_1. m_2. M_1. \\ I_1. I_2. C. P_1. P_2. (P_3.) M_2. M_3. [M_4] \end{array}$$

In the last formula, relative also to man, the elements, whose development is suppressed, are placed in brackets, as is also done in the second formula. In my hypothesis two principles are involved, which will be discussed separately, viz., the belonging of our first permanent molar to the so-called milk dentition, and, secondly, the disappearance of two elements in our permanent set of teeth, the third premolar and the molar originally hindmost. A further consequence of this hypothesis is that the three molars of the catarrhine Primates are not homologous with the three molars of the Platyrrhinae, our second molar corresponding with the first of the latter. I question the nature of our first permanent molar. My opinion is that this tooth is homologous with the third milk molar of the more primitive Primates. Evidently the process by which this tooth became the first molar of our permanent dentition is composed of two factors, to

wit: the loss of its succeeding tooth (the third premolar of the lower forms), and secondly, the fact that an originally deciduous tooth became a persisting element. It is clear that these phenomena stand in a close relation to each other, for a milk tooth cannot acquire the character of a persisting tooth, so long as the evolution of its successor is not suppressed. The two events must have happened simultaneously. As to the question whether the evolution of the milk tooth or the regression of the permanent element was the leading factor in this process, I incline to the first of these two possibilities, on the following ground: I consider that the evolution of the dental structure of the catarrhine Primates commenced with the reduction and final loss of the hindmost molar of an ancestor with a platyrrhine dentition, perhaps in consequence of the shortening of the jaws. By this reduction the grinding surface of the set of teeth underwent a shortening. This circumstance (without importance in small animals such as the Hapalidae, who live principally on soft food or insects) became disadvantageous as the species grew taller and the nature of the food required a larger grinding surface. It is very important that the third milk molar of the platyrrhine monkeys is a larger tooth, with a greater surface and more cusps than its successor, the third premolar. Especially in Hapalidae is the difference notable. And so it was advantageous to the grinding function of the dental arch of the historically succeeding larger forms of monkeys, that the third milk molar with its four or five cusps was not replaced by a tooth with two cusps only. Thus the grinding surface of the dental arch regained at its anterior end what it had lost in an earlier period of evolution at its posterior end.

These considerations lead me to the supposition that in the process of evolution, the alteration of the character of the third milk molar occurred first, the loss of the third premolar being a necessary consequence of it. Because the third premolar was reduced, the third milk molar became a persisting tooth; but because the permanence of this milk molar brought a functional advantage, the evolution of the third premolar was suppressed.

My hypothesis regarding the origin of the dental formula of the catarrhine Primates explains in a very simple manner the otherwise incomprehensible fact that the diminution of the premolars could occur without a gap in the dental arch. And in the post-canine portion of the set of teeth of the higher Primates a diastema is never found. That the continuity of the set of teeth by the above hypothesis was never interrupted, surely does not tell against the justice of it. The foregoing, however, are mere theoretical considerations, let us now proceed to some more practical arguments.

Regarding the embryological evolution of our dentition and the succession of the eruption of our teeth, I believe our first permanent molar was, in an earlier stage of phylogenetical evolution, a deciduous tooth, belonging to the first or milk dentition. In reality this tooth appears (in man, as in all other catarrhine Primates) before the first permanent incisor. And during the nearly two years between the eruption of our first permanent molar and that of our permanent first incisor, the structure of our dentition is identically the same as in young platyrrhine monkeys. During this period of the platyrrhine phase of our dentition, there are three molars immediately behind the canine tooth.

The affinity of our first permanent molar to the set of milk teeth is more clearly shown the moment the first anlage of this tooth appears in human embryos. According to the investigations of Röse, the anlage of milk teeth commences in the ninth week of embryological development. Immediately afterward the germ of the second milk molar is produced by the dental lamina, the latter is prolonged backwards, and the enamel-organ of the first permanent molar is formed. This happens in the sixteenth week of embryological development. Therefore, there is no discontinuity in the succession of the first anlage of the enamel-organs of our deciduous teeth and that of our first permanent molar. A relatively long time follows before the formation of the other teeth, especially of the second permanent molar. The individual is six months old before the dental lamina recommences to grow further backwards and produces the germ of this

molar. The development of the teeth proceeds very regularly up to a well-defined date, but as soon as the formation of the first permanent molar, immediately after that of the second milk molar, has commenced, further development is stopped during nearly a full year. This fact is in complete accord with the fundamental idea of my hypothesis, that our first permanent molar originally was a deciduous tooth, belonging to the milk dentition.

To form a just judgment of the argument advanced it is essential to keep in view the fact that the germ of our first permanent molar emerges from the dental lamina at a much earlier date, not only than that of the immediately preceding permanent tooth, the second premolar, but even earlier than the germ of the first permanent incisor. This irregularity seems very difficult to understand, since the succession in which the germs of the elements of both dentitions are formed by the dental lamina is a very regular one. First the germ of the first incisor appears, afterward that of the second incisor, then that of the canine, and so on. If the generally accepted opinion that our first permanent molar belongs to the second dentition be correct, one must wonder why the germ of a tooth in the middle of the row arises even relatively long before that of the foremost tooth of this row, in contradiction to the general rule. From my point of view, there is not the least difficulty in understanding this phenomenon, our first permanent molar not being a tooth in the middle of the row of the second dentition but the hindmost tooth of our milk dentition. And viewed in this light, the succession in the outgrowth of the different enamel-organs from the dental lamina proceeds regularly.

To this argument, taken from embryology, I will add one of a morphological nature, viz.: the resemblance of our second milk molar to our first permanent molar. As a rule, the form and cusp differentiation of M_1 shows more likeness to those of the second temporary molar than to those of the second permanent molar. The great difference in the type of our first and second permanent molars is never found in comparing M_1 with m_2 . Our first permanent molar always seems to be a reproduction of the second milk molar on an enlarged scale. These



teeth belong together and seem to be one regularly formed functional entity. The variations of these two teeth always bear the same character, in contradistinction to the second permanent molar, whose variability is of quite another nature. This fundamental similarity between the second deciduous and first permanent molar, easily demonstrated in each jaw bearing teeth of normal structure, becomes yet more evident when the milk molar is of a somewhat peculiar form. In such cases, regularly, the first permanent molar repeats this peculiarity and we can say that the second milk molar always follows the scheme after which the first permanent molar is built up. In order to elucidate this relation I give in figures 1 to 4 some reproductions of the upper jaws of children, with somewhat aberrant forms of the crown of the second milk molar. In all cases the first permanent molar shows the same peculiarity as the milk molar. The fundamental cause of this resemblance will not be discussed: for the present it is sufficient to have established this relation. It is a strong argument in favor of my hypothesis and of great value, since between the first and second permanent molars there exists, as a rule, a dissimilarity in cusp-differentiation.

The evolution of the third milk molar of our platyrrhine ancestral form, as expressed by my hypothesis, would have been impossible had not the development of its changing tooth—the third premolar—been suppressed. I firmly believe that this event really took place and shall now advance some arguments proving the justice of this opinion.

It is a well-known fact that a tooth which was lost in an earlier phase of phylogenetical evolution, reappears sometimes as an individual variation of atavistic nature. It would strengthen my hypothesis if unquestionable examples could be supplied of the reappearance of the lost third premolar of our platyrrhine ancestor. This is not a simple matter. One might collect from odontological literature the descriptions of several cases in which the number of the premolars was increased to three, even to four. But additional premolars do not always have the same genetical significance. And to consider each case of three premolars in man as an atavistic variation—a reminis-

cence of our platyrrhine precursor—would surely be an error. Among the cases of a supernumerary premolar we must look for those in which the additional tooth clearly represents the homologue of the third premolar in New World monkeys. The best criterion in this matter is the topographical relation between the supernumerary premolar and the first permanent molar. This relation, of course, must be the same as that between a milk tooth and its successor. As we know, the replacing tooth is situated in the jaw on the inner side and a little behind the tooth to be replaced. And only those cases in which an equal relation between the first permanent molar and the additional premolar exists can be considered as conclusive proof. A very fine specimen of such a variation is given in figure 5, representing the lower jaw of a *Macacus cynomolgus*. The denture is complete, the teeth are still very sound and all of normal form. In the left half of the jaw a supernumerary tooth developed, having nearly the same form as the second premolar, and standing in the jaw on the inner side and a little back of the first permanent molar. In all respects, morphological and topographical, this case represents the typical conditions existing between a milk molar and its successor, and furnishes strong proof that in earlier times the first molar of the catarrhine Primates was a deciduous tooth, replaced by an element of the second dentition. There are in the odontological collection of the Anatomical Museum of the University of Amsterdam several cases of human dentition with an additional premolar, but in none of these is the supernumerary tooth standing on the inner side of the first molar, and therefore they do not illustrate my hypothesis. Yet it should be kept in view that in some, perhaps in the majority of these cases, the position of the supernumerary tooth is a secondary one, the tooth being pushed a little forward by the mechanical influences of the growing jaw.

Still another phenomenon furnishes a strong proof of my theory. It is well known that occasionally our first molar is really replaced by a third premolar. In literature these cases are sometimes described as examples of the so-called third dentition. Now I absolutely deny the possibility of a third dentition, and I

think that the above-mentioned peculiar phenomenon can be explained very simply by my hypothesis. I have in my collection two cases of a replacing third premolar, but regarding only one have I complete data. In this case the first upper molar on the left side of a man of thirty-five was extracted because of pain. Nearly ten months after this treatment the gap in the dental arch was filled by a new tooth, possessing nearly the same form as the second premolar. In this case a replacing tooth was really substituted for the first molar. After the loss of his first molar and the eruption of the third premolar this individual bore on one side of the upper jaw a dentition closely resembling that of the Hapalidae: three premolars and only two molars. It is evident that the pain in the first molar was caused by the third premolar which was unable to push out its predecessor in the physiological painless way.

Summarizing, I believe that the embryology, morphology and anomaly of the denture of catarrhine Primates furnish sufficient grounds to prove that my supposition regarding the nature of our first permanent molar is a correct one. Originally it was a deciduous tooth, like the first and second milk molar, and like these it belongs to the first dentition. Still another proof will follow:

It is clear from my hypothesis that the three molars of the platyrrhine monkeys are not homologous with the three molars of man. Our second permanent molar is identical with the first of the Platyrrhinae, and our third with the second of this group of Primates. Consequently an element equivalent to the third molar of the American monkeys is wanting in our denture. This logical consequence of my hypothesis, throws new light upon a well-known and often discussed anomaly in human denture, viz.: the so-called fourth molar.

A fourth molar is not a rare occurrence in the present form of human denture. But a typical, well-developed fourth molar is very seldom met with. Harrison has found such a one, "full-sized and typical" in an Irish skull. Two years ago, I had more than 30,000 human skulls at my disposal. Among these, collected from inhabitants of Amsterdam deceased during the

last century, there was none with a regularly formed, complete fourth molar, with four or five cusps, but were several cases with a rudimentary fourth molar. Among the African negro race, however, and among apes this supernumerary element is of a more frequent occurrence, and may reach a degree of full development, regularly elongating the row of the three normal molars. From the investigations of Zuckerkandl² we learn the very important fact that the dental lamina in man nearly always exhibits the tendency to form a fourth molar.

The appearance of a fourth molar in man and apes (in the following I shall confine myself to these two groups of Primates), is a wholly incomprehensible fact. Generally one is inclined to declare it an atavistic phenomenon, this variation finding its inevitable explanation in inheritance from an ancestral stock which normally possessed the larger number of molars. If this opinion should be the right one, the human denture must necessarily have evolved from an ancestral form with four molars. But the difficulty arises that among the representatives of the eocene Primate, as yet known, there is none with such an increased number of molars, all possessing only three. Zuckerkandl has emphasized this difficulty, showing the improbability that the fourth molar is an atavistic element. He inclines to the opinion that this extension of our dental arch is of a progressive character. Selenka, after having examined some hundreds of Orang skulls, found a supernumerary molar in nearly 20 per cent of the skulls. This author, also, does not believe that the variation may be judged as an atavistic one, because an ancestor with four molars is entirely unknown. He agrees with the view of Zuckerkandl that the development of a fourth molar in man and apes is a progressive variation, such a tooth not being a reappearance of an element lost at an earlier date of Primate evolution, but having evolved as an entirely new element. This opinion indicates that the distal end of the denture of the higher Primates is in a progressive state of development. I do not concur in that opinion. If the variation were only met with in the Orang, the possibility that Selenka's opinion may be correct, must

² Sitzungsber. Kais. Akad. d. Wiss., Wien, Bd. 100, 1891.

be admitted, for in this Anthropoid the third molar is always a strongly developed element. But a fourth molar, of a rudimentary shape and size, occurs also in man. And with regard to the posterior end of the human denture, nearly all morphologists agree in the opinion, that this end is in a state of retrogression. The third molar, especially in the upper jaw of the white race, is usually of a reduced size and often fails entirely. Selenka himself asserts, that in man this molar is characterized by a tendency to retrogression. De Terra's researches indicate that in the dentition of Europeans this tooth fails in fully 12 per cent. Therefore the hypothesis of Selenka is contradictory. In the Orang the distal end of the denture bears a progressive character, because it produces a fourth molar; in man the same end bears a regressive character, and notwithstanding this fact, it also can produce a fourth molar. I therefore infer that the hypothesis of Selenka brings no solution of the problem and that it presents even more difficulties owing to the theory that the fourth molar in man and apes is an atavistic element.

But as pointed out already, the occurrence of a fourth molar in man, does not warrant a direct phylogenetic interpretation. Even such morphologists as Wilson and Charnock Bradley hesitate to advance such a theory, and adopt Bateson's view that the appearance of a fourth molar cannot well be explained by reference to ancestral forms, since the four-molar type must be regarded as too remote. Therefore these authors believe the supernumerary molar is a variation merely resulting from a dental germ in excess of the normal number, produced by the unusual distally prolonged dental lamina. They therefore agree, in a fundamental point, with the opinion maintained by Zuckerkandl. It is clear that the main cause for the denial of the atavistical significance of the fourth molar, is the fact that we must go back until we reach the common ancestors of mammals, to encounter beings with an increased normal number of molars. And this is an objection of great value, if a true one. But this is not the case. To understand the fourth molar of man and apes as a reversion we need to trace the line of evolution no farther back than to ancestors with a platyrrhine structure of dentition.

My conception of the manner in which man's denture originated from a platyrrhinically constructed ancestral form, obviates all difficulties in a very simple and logical way. Our first molar is the homologue of the third milk molar of our platyrrhine ancestor, our second molar is identical with the first molar of the platyrrhine Primates, our third molar with the second of the latter, and consequently the occasional fourth molar of man and apes, is nothing else than the equivalent of the third molar of the American monkeys.

The development of a fourth molar is also, in my opinion, an atavistic phenomenon. But by my theory we are not obliged to descend to a problematical ancestor, who perhaps existed in the Mesozoïc age and possessed a greater number of molars. As the construction of our denture was derived from a platyrrhine form by the metamorphosis of a milk molar into a permanent element, and the loss of a third molar, we need not go further back in the line of descent than the last phase of human evolution, i.e. to a predecessor with three premolars and three molars. Such forms existed among the eocene Primates. The divergence of the two groups of Primates, the Old World and the New World type from a common stem took place during the eocene period. The New World stem maintained the primitive structure of the denture, save the Hapalidae in which the third permanent molar was reduced and eliminated. The Old World Primates progressed a step further, by the evolution of the third milk molar into a permanent element of the denture. This demonstrates that in man, as established by Zuckerkandl, the distal end of the dental lamina shows a disposition to produce the germ of a fourth molar, which often leads, particularly in the black races, to the development of a real tooth. This is easily understood. The reduction and final elimination of the tooth took place in a period relatively recent. The fact that in apes and especially in the Orang, the supernumerary molar is met with very often, is perhaps indicated by an elongation of the jaws. This circumstance, of course, favors the further development of the latent germ of a fourth molar.

In the foregoing the relation between the denture of the two groups of Primates and the progress of the primitive toward the higher form is explained in a manner differing from that commonly accepted. But I believe that my conception has many advantages. That others are more simple and my theory more complicated, I admit. But even this complication correlates several facts of a diverse nature explaining them in a very logical manner, and this, I believe, increases the scientific character of my hypothesis. Upon this ground I base the accuracy of the views which I have expressed regarding the manner in which the human denture evolved from that of an ancestor with a platyrrhine denture. Perhaps other investigators, acquainted with other facts relating to the odontology of Primates, are able to supply still more arguments in favor of this theory.

SECOND PROBLEM: TO WHICH DENTITION DO OUR MOLARS
BELONG?

The next problem is one confined to the molar region of our denture, the subject of this discussion being the question to which dentition our molars belong, to the first or milk dentition or to the second or permanent dentition.

Opinions do not agree as to the dentition to which our molars belong. Three theories have been advanced. One group of investigators is of the opinion that the molars belong to the first or milk dentition, being therefore elements of the deciduous set without a successional tooth. Another group of authors believe they belong to the second or permanent dentition, being consequently elements of this set without predecessors. And a third group of morphologists asserts that our molars belong to both dentitions, each molar being the result of the fusion of a tooth of the first dentition with a corresponding one of the second. I treat the problem in a somewhat different manner. In the foregoing section I tried to demonstrate that our first molar was originally a milk molar. I therefore feel sure that this tooth belongs to the first dentition, and I am treating the present problem immediately after that of the evolution of our denture, because in the course of this section, I shall deal with

an observation proving the correctness of the theory advocated in the last section, that our first molar was originally a deciduous tooth. But it does not seem superfluous to point out that this doctrine must be confined exclusively to the catarrhine Primates, and that it fails to hold good with regard to other mammals. Therefore when I speak of our molars in the present section, I keep in mind only the second and third.

I do not intend to criticize the different arguments brought up by authors in favor of their theories. The fact that these diverge widely is a clear indication that in this matter no one author has proven the correctness of his view in an indisputable way. Therefore I shall confine myself to the results of my direct investigation.

Originally I was of the opinion that our molars all belong to the deciduous set. This view was based mainly upon the fact that the ontogenetical evolution of our molars occurs in the same manner as that of our deciduous teeth even in the least detail. To comprehend the full weight of this argument I repeat and emphasize the fact that the ontogenesis of the mammalian tooth is a somewhat more complicated process, showing more peculiarities than the usual description indicates. Having first observed these peculiarities in the development of the milk teeth of Primates, and again in that of the molars, it is readily understood that by this agreement I was inclined to the opinion that our deciduous teeth and our molars belong to one and the same dentition. But my opinion of today differs and I shall consider our second and third molars as elements of the second dentition.

This alteration of my view resulted from the fact that the development of our permanent teeth also shows the complications above mentioned, and in this regard there is not the least difference between the elements of our two sets of teeth. This rendered the basis of my opinion worthless. My present opinion has been formed by tracing the supernumerary teeth in the molar region of man.

As already mentioned I have had the rare fortune to be able to examine an extraordinarily large number of skulls. Of these I collected all those with any variation or anomaly in the den-

ture. Hence I possess a great amount of anomalous material for the study of human dentition. The subject of my first research concerned the supernumerary elements in the molar region of man. This research disclosed facts heretofore unknown, leading me to a standpoint with regard to the morphological significance of our molars which is entirely opposite to that once held by me.

My collection of supernumerary elements in the molar region of man, enables me to confirm a well-known fact, viz.: the extraordinary rarity of a so-called fourth molar in the lower jaw of European man, for among this extraordinarily large quantity of skulls, I have found not a single specimen of this anomaly. In his odontography of human races de Terra mentioned only three cases as having yet been described in literature.

In critically examining all the cases of a so-called fourth molar described by different authors, it is evident that under this name supernumerary teeth of a very different nature are included. The comparison of all additional elements in the molar region of man has made it clear to me that it is incorrect to denominate as fourth molars each supernumerary tooth in this region. There are, to distinguish sharply, two species of supernumerary teeth in this part of the denture. In the first group the additional element is situated immediately behind the third molar, prolonging the row of the teeth in a very regular manner. Sometimes it inclines a little to the lingual side of the axis of the dental arch, a point to which we shall return later on. In the second group the supernumerary tooth is situated at the buccal side of the dental arch, either in the corner between the second and third molar, or in that between the first and second.

Do these two groups of supernumerary teeth represent identical anomalies having the same anatomical significance? If only a small number of both types were to hand, it would be a very difficult matter to give a decided answer. The abundant material collected by me, enables me to have a definite opinion about this matter. I feel sure that the supernumerary teeth are not identical in all of these groups, and that they have a varied

anatomical significance. And for the sake of convenience I shall designate the two anomalies differently. A supernumerary element at the end of the dental arch, situated back of the third molar, I shall denominate a distomolar and such a one situated on the outer side of the arch I shall distinguish as a paramolar. There is a current opinion among anatomists that paramolars and distomolars should be considered identical objects. I shall demonstrate in the next pages that this opinion is a false one.

In my collection there are thirteen cases of a distomolar, of which five are on both sides, five on the right side only, and three on the left side only. Of paramolars there are seven instances, four on the left side, one on the right side, and two on both halves of the jaw. Moreover there are two upper jaws with a distomolar on the left side and a paramolar on the right, also one case with a paramolar on the left side and distomolar on the right, and finally there is a preparation, of the utmost scientific value, with a para- and distomolar on the same (left) side. All these specimens are natural preparations. Moreover the odontological collection of the Anatomical Museum of the University of Amsterdam contains a large number of models exhibiting a paramolar and a distomolar also.

As already indicated I have met with no cases of a distomolar in the mandible. Now it should be emphasized that equal rarity of occurrence can be established, respecting the paramolar, for all the above mentioned cases were found in the upper jaw. I do not possess a single specimen of a paramolar in the lower jaw. It is needless to mention that the number of cases collected by me, do not give any indication of the actual occurrence of these supernumerary teeth, for though I have examined nearly 35,000 skulls, these included a large number of aged individuals who had lost their teeth partially or entirely.

Magitot, the renowned French odontologist, has remarked that paramolars are always situated in the corner between the second and third molar. I cannot concur with this statement. In most cases, it is true, the paramolar is situated in the place designated, but in a minority of cases this tooth stands in the corner between the first and second molar. In figure 6 a repro-

duction is given of a bilateral paramolar, alternating with the second and third molar, and in figure 7 a case is reproduced of a paramolar situated in the corner between the first and second molar. In both specimens the supernumerary element stands in its typical place, which it always occupies, alternating with the normal molars.

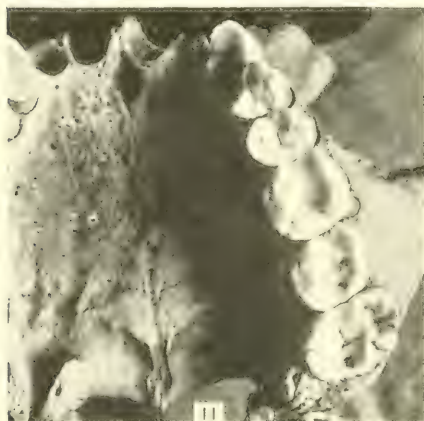
It should be mentioned here that a paramolar alternating with the first and second molars, is not an element identical with that alternating with the second and third molars. Both supernumerary teeth are anomalies of the same morphological order, but the relation between them is one of analogy and not of identity. This statement will be confirmed later on. For the sake of simplified description it is desirable to distinguish these two supernumerary teeth in some simple manner. The tooth alternating with the first and second molar I shall distinguish as Paramolar I, and that alternating with the second and third as molar Paramolar II. As already pointed out, a Paramolar I is of rarer occurrence than a Paramolar II.

We have emphasized above the fact that the paramolars always stand in an alternating position in regard to the molars. Is this topographical relation, which is entirely typical, a primary or a secondary one? The supposition is that in consequence of lack of space, mechanical influences push the supernumerary element into a position, which is the least inconvenient, and yet this is a misconception. We must lay stress upon the fact that the typical topographical relation between molars and paramolars is a primary one. It is impossible to prove the accuracy of this view in a direct manner, such a proof being only obtainable by accidental embryological observation. But in the following pages I hope to be able to justify my assertions in a complete, be it indirect, manner. As we shall demonstrate hereafter, this situation of the paramolars is a factor of much value in the discussion of the ontogenetical nature of our molars.

In literature the opinion is advocated, for instance by Dependorf, that a paramolar, considered intrinsically, should be a distomolar, which during its development has been displaced laterally, from some cause or other, and erupted outside of the



10



normal molar row. As already pointed out, this theory seems to be a plausible one, so long as one has not a sufficient number of specimens of the two kinds of supernumerary teeth at hand to establish indisputable facts. Yet this view is erroneous, as the disto- and paramolars are entirely distinct variations. This can be definitely proved by cases in which both types of variation appear simultaneously in one half of a jaw. I have had the good fortune to meet with one case in which this very rare coincidence occurred. This object is reproduced in figure 8. As can be seen, the first and second molars had fallen out, but fortunately the third remained in place. After the evidence of this specimen, I think there can be no further question about the distinctness of a para- and a distomolar.

A few words may still be said about the anatomical features of the paramolar. In most cases this element is of a very simple shape. The crown never exhibits more than two small and slightly developed cusps. In many specimens the crown has a flattened surface, exhibiting in the center an insignificant depression. In all the cases, from which I formed my conclusions, there was but a single root.

The above named peculiarities will suffice with regard to the anatomy, topography and morphology of the paramolars. Some remarks may still be made regarding the distomolars. In many cases this supernumerary element is situated with regularity behind the third molar, but among my specimens there are some in which it is displaced a little. It is very important to note that in case of displacement the distomolar deviates in a palatal direction as illustrated in figure 9. This fact merits especial notice in view of anomalies in the cusp differentiation of the third molar to be described later on. This tendency to displacement is, of course, due to the pressure of the soft parts and may not be considered as the expression of a primary topographical disposition. The outer aspect of the distomolars is very different. It has been mentioned already that among the specimens investigated by me none occurred in which the distomolar repeated in all respects the form and size of a fully developed normal molar. It appeared usually as a small tooth with

a conical crown, as shown in figure 9. Sometimes it was of an increased size, as in figure 8. I have never found it with more than a single root.

So far we have only discussed the occurrence of a supernumerary molar as an entirely independent tooth outside of or behind the row of teeth. We shall now consider these anomalies from quite another standpoint, as these supernumerary teeth, paramolars as well as distomolars, occur more frequently coalesced with one of the molars, and for the sake of a well-founded judgment respecting the phylogenetical significance of the supernumerary elements in the molar region of man, it is necessary to examine more closely the occurrence of coalesced paramolars. Some points hitherto dark will be cleared up by this examination, and unexpected new facts will be brought to light. We shall discuss the concrescence of the paramolars and of the distomolars, separately, beginning with the former, treating the upper first and thereafter the lower molars.

In case of the coalescence of a paramolar with a molar it is obvious that the supernumerary element unites with the buccal surface of the normal tooth, and appears as an additional tubercle at this side. Such supernumerary tubercles are mentioned in literature frequently, but their identity with independent, free paramolars has never been suggested. De Terra alone, calling such supernumerary tubercles at the buccal side of the molar-crown simply buccal cusps, considered them as a small supernumerary tooth, united with the normal molar. In order to express their developmental significance, I shall distinguish these tubercles as 'para-molar cusps.'

This tubercle shows very different degrees of development. In case of the most complete development it appears as an appendage to the normal tooth with a small free crown and a proper root, being attached to the molar only by means of its neck, as illustrated in figure 10. Such cases, in which the primitive features of the paramolar are wholly recognizable, are very evidently intermediate forms between a free paramolar and a simple paramolar tubercle. Such cases however are very rare.

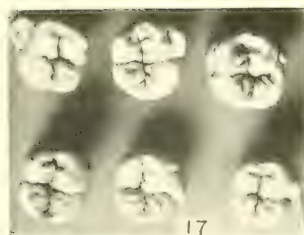
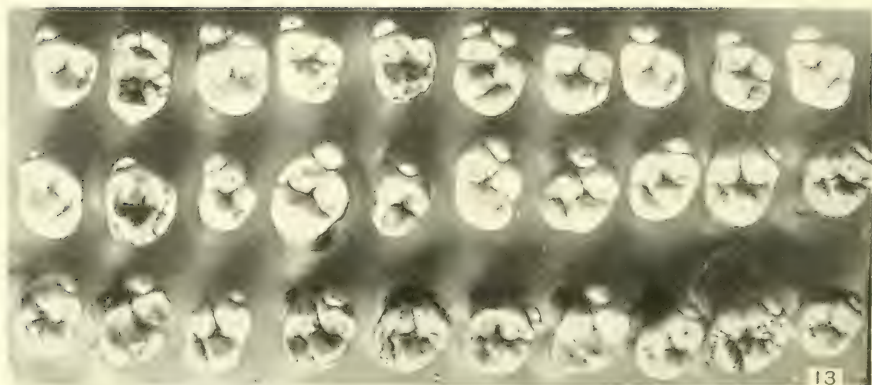
These occur most frequently in which the supernumerary element is reduced to an additional cusp at the buccal side of the crown, the root being completely fused with the anterior buccal root of the molar. To procure a general idea of the form of molars provided with a paramolar cusp, I have reproduced in figures 11, 12, 13 and 14 some preparations from my collection. In figures 11 and 12 is reproduced a maxilla, the third molar of which bears the indisputable signs of a coalescence with the Paramolar II. In figure 13 a series of thirty second molars with a paramolar tubercle are represented and in figure 14 a series of twelve third molars with such an additional cusp. One might look in vain in any odontological collection for series as fine as these, which undoubtedly are unique.

As clearly shown by these figures, in most specimens the coalesced paramolar exhibits a single cusp, united with the crown of the molar in such a manner that there remains no doubt as to its nature as an accessory element, foreign to the normal cusp-differentiation of the molars; for the normal feature of the crown is not altered by this supernumerary cusp, which never is united with the crown in such a manner that a grinding surface of higher differentiation results. It never participates in the function of the molar.

In some cases, relatively rare, the paramolar-cusp is of a larger development, possessing two tubercles and very rarely it happens, as in figures 11 and 12, that the additional element bears the character of a little crown, with a central depression.

A close study of all the molars with a paramolar tubercle now in my possession, has acquainted me with its three following characteristics. First: a paramolar-cusp is never found in the first molar; second: this supernumerary cusp occurs more frequently in the second molar than in the third; and finally: the paramolar-tubercle is always united with the anterior buccal cusp of the molar (second or third).

These three peculiarities merit our careful attention. The lack of the cusp in the first molar is a fact already established by de Terra and Batujeff. Upon a close examination, I have seen, in the course of my investigation of skulls, at least twenty



thousand first molars. And in none of all these, have I met with a vestige of a paramolar cusp. This fact is very important, not only as an unexpected peculiarity, but as furnishing a new proof of the correctness of my conception of our first molar as a tooth which was originally a milk molar. This will be made obvious in the discussion of the significance of the morphological phenomena we are about to describe.

The fact that a paramolar-cusp occurs less frequently on the third molar than on the second is not difficult to explain. As pointed out above, the opinion advanced by Magitot that a paramolar is always situated in the corner between the second and third molars is incorrect. To prove this, a reproduction was given of a maxilla exhibiting an additional tooth standing in the corner between the first and second molars. This phenomenon induced me to distinguish a Paramolar I and a Paramolar II. The former, alternating with the first and second molars is less frequent than the latter. By combining all known facts, we are able to establish the following coincidence: a rare Paramolar I and a more frequent paramolar tubercle on the second molar; and further a more frequent Paramolar II and a lesser frequency of a paramolar tubercle on the third molar. The relationship between these facts is not difficult to conceive. The first paramolar coalesces with the second molar and the Paramolar II with the third molar. Now the facts enumerated enable us to deduce that the Paramolar I shows a greater tendency to coalesce with its adjacent molar, whereas the Paramolar II shows more inclination to remain independent, hence the difference in the occurrence of the paramolar tubercle in the two molars. To get a correct idea of the frequency of the paramolars, it is therefore necessary to compile all the cases of a free Paramolar I with those of a paramolar tubercle at the second molar, and to do the same regarding the Paramolar II and the paramolar cusp on the third molar. The result of this will show that the frequency of the two supernumerary elements is nearly the same, with perhaps a small preponderance of the Paramolar II.

We have now arrived at the third peculiarity mentioned above, viz.: the fact that the paramolar cusp, without exception, is

attached to the anterior buccal cusp of the molar. A close look at the molars represented in figures 11, 12, 13 and 14 suffices to confirm the accuracy of this statement. I never saw a case of a paramolar tubercle arising from the posterior buccal cusp of the molar. If the tubercle is strongly developed, and, as sometimes happens, appears as a double cusp, one of these two is displaced a little more distally, approaching the fissure which separates the anterior and posterior cusps. Still, even in the rare cases represented in figures 11 and 12, in which the con-cresced paramolar was very strongly developed, it manifests its close relationship to the typical cusp of the molar-crown.

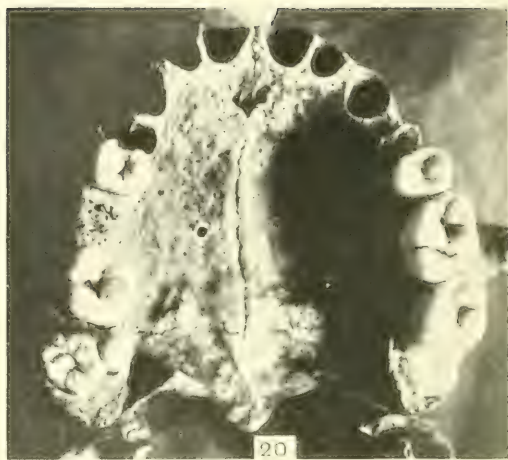
What do we learn from this very interesting, sharply defined topographical affinity of the paramolar tubercle to the molar? In one of the foregoing pages much stress was laid upon the situation of the paramolars in the corners between the molars, and it was emphasized that this situation cannot be considered a secondary one, because it is primary, the paramolars evolving in alternation with the molars. At the place designated I omitted to furnish positive ground for this statement. I now believe, that the above established fact of the sharply confined topographical relation between the coalesced paramolar and the molar fills up this lack in a decisive manner. For it is needless to discuss in detail that such a union as occurs between a molar and a paramolar cannot be effected secondarily. The normal and the additional element must have been closely connected with each other from the earliest phase of development: the dental papilla of the paramolar must have been fused with that of the molar from the beginning. And by the regularity with which the paramolar cusp appears as an appendage of the anterior buccal cusp of the molar, it is established unquestionably that the dental papilla of the paramolar, from its origin, is situated in close proximity to the mesial border of the molar, or in other terms, that this papilla alternates with those of two molars. This is a very important statement, being one of the leading views in the discussion about the developmental significance of our molars. Now arises the question why the paramolar, alternating with the molars, always coalesces with the molar situated

distally from it, and never with that mesially. The solution of this problem is a very simple one. However, to take up the question now would be a digression and I therefore prefer to return to it at a more suitable place.

In the foregoing, the principal points concerning the paramolar cusps in the upper jaw are established, and we shall now continue examining the corresponding occurrences in the lower jaw. I recall that never have I found a free paramolar in this jaw, a fact evidently harmonizing with the rule that supernumerary teeth in the mandible are commonly much more rare than in the maxilla. On the contrary I have met with several coalesced paramolars in the lower jaw.

In comparing the usual features of the coalesced paramolar in the lower and upper jaw with each other, one is impressed by the remarkable difference in the behavior of this supernumerary element in the two jaws.

When a paramolar-cusp occurs in one of the lower molars, this tubercle frequently proves its original significance as an independent tooth by the possession of a normal root. In the upper jaw this is a very rare occurrence. I shall term this root 'paramolar-root.' Now in the lower molars often only the paramolar-root is present whilst the tubercle, i.e., the crown of the paramolar, is entirely absent. In the upper jaw on the contrary, just the reverse happens. Here the paramolar tubercle is often found without any vestige of a root, the latter being entirely fused with the anterior buccal root of the molar. In case of conerescence in the upper jaw the paramolar manifests itself usually by its crown, as the paramolar-cusp in the lower jaw, on the contrary, by its root. I have no theory whatever regarding the cause of this singular difference in the behavior of the upper and lower paramolars. In figure 15 some twenty lower molars are represented, provided with a paramolar root in different degrees of development. In the top row no paramolar tubercle is to be noted, yet we observe how the paramolar root gradually becomes longer and stronger, until in the last molars of this row it is as long as the two normal roots. The second row is composed of seven molars in which the paramolar root is even



more strongly developed than in the top row. This root is seen to be situated on the buccal surface of the tooth, starting from the crown as an independent root. In the last two objects of this row, the first indication of a paramolar tubercle in connection with the root appears. Finally in the bottom row seven molars are reproduced with a fully developed paramolar-root and a more strongly developed tubercle. In more closely observing the objects of this row, all doubt as to the relation of the paramolar root and the paramolar cusp is wiped out.

Except for the difference pointed out between the coalesced paramolars in the upper and lower jaw, these elements agree in every respect, as I have never seen a single vestige, either cusp or root, of a paramolar tubercle on the first lower molar. Furthermore, in the lower as well as in the upper jaw the paramolar is coalesced only with the anterior buccal cusp of the molar. This may be observed in the objects reproduced in figure 15. This anatomical relationship is demonstrated still more forcibly by figures 16 and 17, in which a series of second and third molars is reproduced, seen from above. It is needless to further elaborate these points, as they are fully discussed in the preceding pages.

Before leaving this subject, I must return briefly to the second form of supernumerary tooth in our molar region, viz.: the distomolar. As pointed out already this supernumerary element, as well as the paramolars is sometimes coalesced. It is obvious that such a coalescence only can take place with the third molar. A few words upon the character of this coalescence.

On one of the preceding pages stress has been laid upon the fact that a distomolar is not always situated regularly behind the third molar, but is slightly displaced now and then, and in case of such a displacement the distomolar is always shifted in the palatal direction. Now I suppose that originally a distomolar is always situated more or less disto-palatally from the third molar, for in case of coalescence of this supernumerary element, the fusion is always effected with the palatal side of the third molar. This is demonstrated by figures 18, 19 and 20. In figures 18 and 19 a lower third molar is represented, at the

lingual side of which an accessory tubercle protrudes. This tubercle may be termed a distomolar cusp because it is nothing else than the distomolar fused by means of its root with the normal third molar. In figure 20 a corresponding case in the upper jaw is represented. By its entirely different situation it is very easy to distinguish a paramolar from a distomolar cusp, for as pointed out, the paramolar is always in close relation with the anterior buccal border of the molar, whereas the distomolar is fused with the palatal side of the same.

After this brief description of the additional elements occurring in the molar region of man we return to our starting point. The purpose of the research for typical additional teeth in the molar region was to collect sufficient data for a personal judgment as to the nature of our functional teeth. For it is clear in connection with this problem that no hypothesis is acceptable, except one in which all derivatives of the dental lamina are reckoned with; and I hope to have demonstrated that the paramolars, though anomalous elements in human denture, are not unusual products of this lamina, without any developmental significance, but that they are regular elements. They must be considered as teeth which functioned at one time in an earlier, long-past phase of the evolution of human dentition, but afterwards became rudimentary and were eliminated from the molar row. And, considering that, save in some Marsupials, the number of molars in all recent mammals does not surpass three, these paramolars evidently represent teeth which functioned in our ancestral forms of the mesozoic period.

The possibility that they are only casual products of the dental lamina must be firmly rejected; for their topographical situation (their relationship to the normal molars), is of such regularity and invariability, as can only occur with essential, originally normal products of the dental lamina, formerly produced on well defined spots, in accordance with the other elements. And in the making up of a scheme of all teeth produced by the molar region of our dental lamina, the paramolars require to be considered as elements of equal value to the molars.

After this statement we shall try to solve the question as to the place occupied by the paramolars in our dental system. The principal point to reckon with in outlining such a system undoubtedly is furnished by the fact that the paramolars alternate with the molars, the Paramolar I with the first and second, the Paramolar II with the second and third. In a clear manner this fundamental point is expressed by the following simple scheme in which the paramolars are represented by the symbol Pa, the molars by Mo.

| | | | | |
|--------|-------|--------|--------|--------|
| | Pa I. | | Pa II. | |
| Mo. 1. | | Mo. 2. | | Mo. 3. |

This scheme recalls at once the topographical relationship between the elements of the first and second dentition. For these are also placed in two rows, an outer and an inner. The teeth of the inner row, i.e., of the second dentition, develop lingually from those of the milk dentition, and moreover their topographical relation is such that the permanent teeth alternate with those of the milk dentition. So the first permanent incisor alternates with the first and second milk incisor, the second permanent incisor with the second incisor and the canine of the milk dentition, etc. A milk tooth consequently is succeeded by a tooth originally situated disto lingually from it. In the farther progressed phase of development of our dentition, this primitive topographical condition is disturbed, so that for instance the canine frequently erupts at the outer side of its milk predecessor.

In the next scheme a simple representation is given of the original relation between the elements of our first dentition, and the corresponding ones of the second.

| | | | | | |
|----------------|----------------|---|----------------|----------------|--|
| i ₁ | i ₂ | c | m ₁ | m ₂ | |
| I ₁ | I ₂ | C | P ₁ | P ₂ | |

A comparison of this scheme with that of the molars and paramolars, immediately reveals the agreement of the two with each other, the paramolars being situated with regard to the molars, just as the milk teeth are with regard to the permanent teeth.

This agreement is such a striking one, that I believe it to be the manifestation of a fundamental relation between the paramolars and the elements of the first dentition. I think the paramolars are the continuation of the elements of the milk dentition which, however, during the progress of the evolution of the mammalian dentition, grew rudimentary and finally were eliminated from the row of functional teeth. Their reappearance in man must be ascribed to retrogression. But in order to prevent misunderstanding, I wish to emphasize that the elimination of these teeth took place at the root of the mammalian stem. Perhaps their homologues may be found still as functioning teeth in some marsupials, with an exceptionally increased number of molars. But I shall not dwell on this point. By my conception of the morphological value of the paramolars, the question whether our molars belong either to the first or to the second dentition is solved at the same time. For if the paramolars belong to the row of milk teeth, necessarily the functional molars must belong to the second dentition, the set of permanent teeth. One must not forget, however, that this statement holds good only for the second and third molar, the first belonging, as fully demonstrated in the foregoing pages, to the set of milk teeth. The whole set of the so-called first dentition is therefore composed in man of the following elements: the five deciduous teeth, the first permanent molar, and the two paramolars which appear only as individual variations.

Arrived at this point of view, we are able to explain a phenomenon, upon which stress is laid above, viz.: that never does a first molar either in the upper or in the lower jaw show a paramolar cusp or a paramolar root. This total absence of an additional cusp or root on these teeth appearing not very rarely in the second and third molar, is readily understood. The paramolars are elements of the outer set of teeth, the so-called first or milk dentition. They may fuse occasionally with the elements of the inner row, manifesting themselves as a supernumerary cusp or root. But our first permanent molar itself is an element of the outer set of teeth, therefore a paramolar tubercle on this tooth is a quite unimaginable thing.

As mentioned above the question whether our molars belong to the first or the second dentition is answered by authors in very different ways. Some say they belong to the first row, others assert they are elements of the second row, and finally a third group consider our molars a result of the coalescence of a tooth of the first with one of the second row. I agree with none of these opinions. In the first pages I have tried to make it clear that the first molar of the catarrhine Primates, and therefore also of man, was originally a milk tooth, and in the present section, I attempt to demonstrate that the second and third molars belong to the second row, the corresponding elements of the first row being the two paramolars. My conception is, I admit, a somewhat more complicated one, than those of other authors. But I can say of it, what scarcely may be said of the other conceptions to the same degree, that my hypothesis is not a mere theoretical one, being founded upon a great variety of facts partly resulting from the examination of an extraordinarily large amount of material. Therefore my hypothesis bears the character of a conclusion and not that of a postulate. Moreover it demonstrates, at the same time, anomalies which cannot be explained satisfactorily by other means.

Before entering into the third problem which we intend to discuss in this essay, viz.: the future changes in human dentition, we shall try to combine the results of investigations treated in the foregoing sections. The nature of the subjects treated in these sections facilitates a discussion of the same from a common point of view; for both concerned a phase of the phylogenetical history of our dentition. In the first, in which I tried to throw a new light upon the relationship between the dental formula of platyrrhine and catarrhine Primates, particular attention was drawn to the significance of our first molar. Several points were elaborated to prove that in the ancestors of man this tooth was a milk molar. In the second section I entered into the problem of the nature of our second and third molars, as belonging to either the first or the second dentition. I consider the demonstration of a rudimentary region of our milk dentition, extending buccally from our second and third molars

and exhibiting itself by the occasional development of two or perhaps three rudimentary teeth which alternate with the normal molars, the principal result of this examination. Therefore the subjects of the two sections show a common feature, concerned with some question about the phylogenetical development of the molar region of man. This common characteristic makes the combination of the results of both investigations possible. In doing this we get a more complete insight into the natural structure of our dentition. In the most compendious manner, such a combination is given by a scheme in which the elements of both dentitions are represented in their topographical relation to each other. The knowledge of such a scheme is desirable, as a starting point for further investigations, after the subsequent changes in our dentition. For, as we hope to demonstrate in the next paragraph, changes identical with those which have taken place in the past, will occur again in the future.

I shall begin by recording once more the dental formula of the Platyrrhinae and Catarrhinae, in their natural relations:

Dental formula of Cebidae:

$$\begin{array}{c} i_1. i_2. c. m_1. m_2. m_3. \\ I_1. I_2. C. P_1. P_2. P_3. M_1. M_2. M_3. \end{array}$$

Dental formula of Hapalidae:

$$\begin{array}{c} i_1. i_2. c. m_1. m_2. m_3. \\ I_1. I_2. C. P_1. P_2. P_3. M_1. M_2. [M_3] \end{array}$$

Dental formula of Homo:

$$\begin{array}{c} i_1. i_2. c. m_1. m_2. M_1. \\ I_1. I_2. C. P_1. P_2. [P_3] M_2. M_3. [Di] \end{array}$$

The teeth reduced and lost, during the progress of human evolution, are placed in brackets. The so-called fourth molar in man, which, as demonstrated in the first section, is homologous with the third molar of the platyrrhine monkeys, is indicated as Distomolar (Di). In the three formulas, expression is given to the natural topographical relationship of the elements of the two dentitions to each other, by the alternation of the

symbols of the elements of the first and second dentitions. It is needless to give any further explanation of these formulas, the same having been discussed in detail in the first section.

In the next formula the rudimentary elements in the molar region of man, described in the foregoing paragraph, the Paramolars I and II—are added.

$$i_1, i_2, c, m_1, m_2, M_1, [Pa_I] [Pa_{II}] \\ I_1, I_2, C, P_1, P_2, [P_3] M_2, M_3, [Di]$$

This formula represents the construction of a nearly complete human dentition. In it all elements, really existing or lost dur-

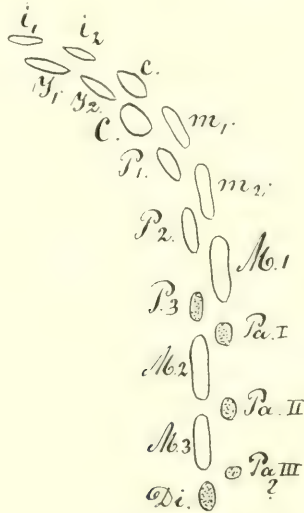


FIGURE 21

ing the last phase of human evolution, are mentioned and placed in their natural topographical position. The relation between the elements may perhaps appear still more clearly, by a scheme given in figure 21. In this scheme the lost teeth are made recognizable by dotting. As mentioned, this scheme is not an absolutely complete one; for we know that during the development of the primate stem, probably in the eocene ancestors of man, an incisor and the first post-canine tooth are reduced and suppressed, three being the primitive number of incisors

and four that of the premolars. However it is not my intention to enter into the questions surrounding these lost components of primate-dentition, but to confine myself to the molar region.

It is very remarkable that in the scheme of our dentition, drawn up in figure 21 (both rows), both the first and the second dentitions are complete. Each tooth of the inner row (or second dentition) is situated opposite an interstitium dentale of the outer row, i.e., the milk dentition. The lost third premolar corresponds with the dental interstice between the first permanent molar (i.e., the third milk molar of our ancestors) and the Paramolar I, and the second molar alternates with the Paramolar I and Paramolar II.

The scheme shown in figure 21 demonstrates, in a very convincing manner, that only in considering our first permanent molar as an element of the outer row do both dental arches appear complete and without interruption. This fact is considered by me as a strong proof in favor of the correctness of my views regarding the manner in which human dentition evolved from that of a platyrrhine forefather.

In the second paragraph stress has been laid upon the absolute absence of a paramolar tubercle or a paramolar root on the first permanent molar of man. This absence is so typical, that it may be regarded as a characteristic morphological feature of our first molar. The cause of this peculiarity, briefly indicated previously, becomes intelligible at first sight in looking at the scheme of figure 21. The paramolar cusp of the second and third molar represents the rudimentarily developed Paramolars I and II of the outer row, coalesced with the second or third molar. The first molar, however, is itself a component of the outer row, and a tooth never develops at the buccal side of the same. It is impossible, therefore, to find an additional buccal cusp on this tooth. I may be permitted to repeat that this fact also emphatically proves the correctness of my opinion as to the odontological significance of our first molar.

Furthermore, by the scheme in figure 21, the following question suggests itself. In the first section it is demonstrated

that the fourth molar of man, or his distomolar as I term it, is homologous with the third molar of the platyrrhine monkeys. This tooth, being a real molar, belongs therefore to the inner row. And now the question arises whether there can still be developed a tooth corresponding with the *interstitium dentale* between the third molar of man and his distomolar. Such a tooth would represent a third paramolar. In the scheme of figure 21 this element is drawn at its theoretically deduced place, but, being of dubious occurrence, is indicated by a mark of interrogation. Considered from a merely theoretical point of view, the occurrence of such a rudimentary tooth is not at all an unthinkable possibility. But in man it is very difficult to recognize such an element practically. For, suppose, in some one, it should really be developed, it would then be situated as a rudimentary tooth behind the third molar. But the distomolar possesses an identical position. Therefore it would be very difficult to distinguish an eventually occurring third paramolar from a distomolar, both teeth being developed rudimentary elements, situated behind the third molar. There is only one instance to prove the occurrence of a third paramolar in man indisputably, viz.: the occurrence of two rudimentary teeth behind the third molar. Such a case is recorded by Turner.³ In an Australian aboriginal skull, this author found two sockets behind the left upper wisdom-tooth in each of which a supernumerary molar was contained. It seems very probable to me, that one of these was the distomolar and the other the Paramolar III.

In examining the individual variations in lower monkeys, the third paramolar appears not merely as a theoretical conception: for in platyrrhine monkeys with the original number of three molars, all belonging to the inner row, a supernumerary molar sometimes appears, corresponding with the *interstitium dentale* between the second and third molar. A very fine specimen of this interesting variation is described and represented by Bateson in his "Materials for the Study of Variation," p. 208. The

³ Turner, W. An Australian skull with three supernumerary upper molar teeth. *Journ. Anat. and Phys.*, vol. 34.

learned author cannot decide as to the origin of this extra tooth standing outside the arch. He hesitates between the possibility that the extra tooth originated by a division of M_2 and the possibility that it represents an addition to the normal series. I believe the third possibility, i.e., that this supernumerary element represents the hindmost element of the outer row of teeth (a third paramolar), is much more evident.

Returning again to the scheme given in figure 21 a final remark may be made concerning the lost third premolar of man. As shown clearly by the given scheme, this tooth alternates with the M_1 and Pa_1 of the outer row. The reappearance of this tooth as an atavistic variation in man is a very rare event. However, it seems to me that this tooth causes an anomaly occurring frequently in the lower first molar of man. It is pointed out in the second section, that in the lower jaw the paramolars, in case of coalescence with the second or third molars often appear as a supernumerary root at the anterior buccal corner of the molar, contrary to the upper molars in which the paramolars most frequently are found as an additional tubercle coalesce with the anterior buccal cusp of the molar. Now in the first lower molar a corresponding peculiarity, such as that in the second and third, occurs occasionally. In this tooth also an additional root sometimes is present, however, not at the outer side as in the second and third molars, but at the distal corner of the inner side, i.e., lingually from the distal root. Perhaps this warrants the supposition that this additional root is not an extra root at all, but simply the result of a division of the normal posterior root. This supposition is, however, an erroneous one. Out of 1800 first lower molars of man I have collected eighteen instances of a supernumerary posterior root as described, and a comparison of these specimens proves that there is no question about any division of the posterior root. For if such really was the case, undoubtedly the collected specimens must show the supposed division in different degrees of perfection, beginning with a simple bifurcation of the apex of the root and continuing until a complete reduplication appears, as seen in other cases of the division of roots of teeth. But there is not the least indi-

cation of such a process of progressive division. In all specimens, the additional root, be it large or small, is wholly independent of the normal posterior root. Even in its smallest development it denotes its character as an additional element as unmistakably as the so-called paramolar root does on the second and third molar. Therefore I feel sure that this extra root of the first lower molar in man is the manifestation of an element in earlier phases of evolution wholly independent of the first lower molar, as well as the paramolars. However this element did not develop at the outer but at the inner side of the first molar. And in critically observing the scheme in figure 21 it becomes evident that in case of the coalescence of a rudimentary P_3 with the adjacent tooth, it must unite itself with the posterior part of the lingual side of the first molar. Thus the topographical relationship between the lost P_3 of man and his M_1 is in full accord with the significance of the supernumerary root on our first molar, as described above. Therefore I wish to denominate the extra root of this molar as 'radix premolarica.'

THIRD PROBLEM: ON THE PROGRESSIVE VARIATIONS IN HUMAN DENTITION

The third problem with which we shall occupy ourselves in this essay, concerns the modifications which will evolve in the future in human denture. These changes are of different natures, viz.: changes in the form of the teeth and changes in the number. I shall confine myself to the discussion of the last kind of variations.

Several authors, projecting a classification of the anomalies in the number of human teeth, have pointed out that two groups of this kind are to be distinguished. A first group contains the variations of an atavistic nature, whereas the second group contains the variations of a progressive character. We must consider as progressive variations all those by which the human denture differs more widely from that of its forerunners and therefore also differs from the normal denture of the man of today. I agree with such a classification. But I do not believe that every anomaly occurring in the human denture must necessarily

be either of an atavistic or a progressive nature. Besides these a third group ought to be distinguished, containing the variations in the structure of our denture which have nothing to do with its developmental history, either in the past or in the future. These anomalies are the results of a deviation in the ontogenetical evolution of one of our teeth, the cause of which is not of a hereditary nature. Undoubtedly this kind of variation occurs in the denture of man as well as of other mammals. It will be readily understood that a pathological process, occurring in the neighborhood of a tooth during its development, may have an influence upon this organ, when fully formed. And it is believable that by such a pathological cause, even the number of teeth may be influenced. Now there are authors who are inclined to absolutely deny the occurrence of atavistic variations in human denture, simply because there is a group of anomalies which definitely lack any developmental significance. I do not agree with this opinion. I admit, it is often very difficult to decide at first view whether a variation belongs to this latter group or not. The fact that we still possess an incomplete knowledge of the developmental history of our denture must certainly be considered as the principal cause of this difficulty. But just this lack of sufficient knowledge must be a stimulant to penetrate more deeply into the mystery of this part of our phylogenetical development, and to try to discover facts until now unknown, which will enable us to build up this history in a more complete manner. To accomplish this we must first undertake as completely as possible a systematic investigation of all kinds of variations occurring in human denture. In this manner only shall we be able to discern the atavistic and progressive variations from those without any phylogenetical significance. However this task is not an easy one as may be illustrated by the following simple example.

One of the most common anomalies of our denture is the appearance of a supernumerary incisor in the upper jaw. Now it may be taken for granted that one of our ancestral forms possessed three incisors as a normal condition, and, taking this fact into consideration, one must incline to the view that a

third incisor in man should always be considered an atavistic phenomenon. Such a conception however, would be, by its frequent occurrence, an error, the increased number of our incisors resulting from different causes. After an ample examination of this anomaly in the denture of man and other Primates, it has become clear to me that only the supernumerary incisor arising close to the median line of the palate is an atavistic one. This supernumerary tooth is usually of a conical form, standing most frequently inside the arch. The other supernumerary incisors, nearly always standing regularly in the arch, and in most instances possessing a normal incisiform crown, are not at all of an atavistic nature. They are instances of schizogenic variations, as I may call them, because they originate by a division of one of the two normal incisors. They are instances of reduplication without any historical significance. But, it is an error to deny the historical significance of all variations, simply because there is a group in which the occurrence is due to another factor. I agree fully with the statement of Professor Wilson:⁴ "It is beyond question that numerous instances of variation are of purely 'teratological' significance, and the existence of these need not be allowed unduly to discount the value of others which are almost inevitably suggestive of reversion." There are authors of the opinion that all variations or irregularities in human denture, without developmental significance, are symptoms of degeneration, a view with which I cannot concur. If one considers these anomalies as symptoms of degeneration, the same must also be done with regard to all anomalies occurring in the other anatomical systems of our organism, the evolutionary nature of which likewise cannot be demonstrated. And certainly there is no morphologist, who will accept this conclusion. I will illustrate this with an example. The sternalis muscle is a variation occurring not infrequently in man, being present in 4.4 cases out of 100. Undoubtedly this occasional muscle cannot be an atavistic one, because it never participates as a normal element in the muscular system of any mammal. That it should be considered a variation of a progressive nature, is equally doubt-

⁴ Journal of Anat. and Phys., vol. 39, p. 128, 1905.

ful, because, even in case of strong development the muscle lacks a special function. But it is not permissible, I think, upon these grounds to maintain the view that the sternalis muscle is a symptom of degeneration. Its existence is simply caused by a factor of an unknown, perhaps of a mechanical nature, working upon the mass of embryonic tissue from which the group of pectoral muscles differentiates. Beyond this the real nature of this factor may remain open to question.

Returning to our special subject, I wish to give a brief account of the variations of the human denture of today, which seem to me to prepare for the construction of the future human denture. From this point of view such variations may be distinguished as progressive ones, even though their essential character is that of morphological retrogression.

The examination of the abundant material showing dental anomalies present, in the Anatomical Museum of the University of Amsterdam, has convinced me that the principal change which will occur in the future will be the continuation of the process which took place in the past and with which we became acquainted in the first paragraph.

The fundamental characteristic of the developmental history of the human denture is the diminution in number of the constituent elements. This process is not limited to a single point of our denture but proceeds at two points at least, viz.: in the incisor region and in the molar region. I shall first consider the reduction of our incisors.

One of the most generally known indications of reduction in our denture is that of the second incisor in the upper jaw. Often this reduction is noted in several generations and members of a family, so that in one family all degrees of reduction, and total absence also, occur simultaneously. According to the investigation of Röse, the anomaly occurs in about 2 per cent of the German people. This author emphasized the fact that his investigations indicate no relation between the frequent absence of this tooth or its reduction, and the shape of the facial skull. In long-faced individuals the anomaly is as frequent as in broad-faced ones.

It is a well-known fact that the eocene representatives of the primate stem possessed three incisors in both dentitions. During the progress of primate evolution one of these was suppressed. The genus *Adapis* forms a link between the primitive and the recent condition, still possessing three incisors in the first, but only two in the second dentition. Which incisor has disappeared is a much debated point, upon which authors do not agree. The literature on the subject shows that each of the three primitive incisor teeth has been claimed as the defaulter. But I do not wish to enter into this problem, confining myself to the statement, that I think it must have been the first or that most mesially situated. This conviction is based upon the fact that an additional incisor of conical shape not infrequently occurs in man next the median plane behind the central incisor. From the observations of H. B. D. Lieka⁵ this supernumerary tooth occurs, curiously enough, more frequently in Indians than in Whites. This author also believes these supernumerary elements in the intermaxillary to be reminiscences of conditions which have existed in past ages in mammals that were the ancestors of the Primates. The changes occurring in the series of incisors, therefore, are of a somewhat special character. In earlier times a diminution in the number of these teeth took place by the loss of the most mesially situated, in both jaws; and in man of today the decreasing process is progressing, but differs from the first phase of reduction in two regards. First, it is not now the first but the second incisor, and secondly the reduction is confined to the upper jaw. When, in the future, the loss of this second incisor has become the normal condition, the singular condition will result that in a primate the construction of the set of teeth in the two jaws will be different, showing three incisors in the lower jaw and only two in the upper.

This progressive variation in our denture is briefly recounted here, because it is one of those most generally known and therefore it should be mentioned in a discussion upon the developmental history of our dentition. Nevertheless it is by no means the most interesting.

⁵ Human dentition and teeth from the evolutionary and racial standpoint. The Dominion Dental Journal, 1911.

Besides the reduction in the incisor region of the upper jaw there is still another, also well-known in the molar region of both jaws, viz., the reduction of our so-called wisdom teeth. This process is much more important, because it is a repetition of the homologous process which had taken place in an earlier period of evolution.

The absence of the third molar is by no means uncommon. According to de Terra it is failing in about 12 per cent of the European races. Concerning this reduction my opinion differs somewhat from that commonly accepted. I believe the reduction of our hindmost molar to be connected with another occurrence of quite a different nature which concerns our second milk molar and its successor, the second premolar. It may happen, for instance, that the second milk molar is not shed, but persists during a long period of human life, while the second premolar never appears at all. This phenomenon is, contrary to the current opinion of practitioners, a physiological and not a pathological one, being the expression of a normal evolutionary tendency. The grounds upon which this statement is based, will be given in the next pages.

It is very interesting that, while the reduction of our third molar is a fact of general note, the persistence of our second milk molar has scarcely arrested the attention of morphologists. So far as I know the question as to an eventually evolutionary significance of this anomaly has never been asked. It is the common opinion of practitioners that a persisting milk molar is to be regarded as a case of irregular or wholly impossible tooth-changing caused by an impediment, without any developmental importance; otherwise it has been maintained that this persistence is only due to lack of space for the permanent tooth. This statement is entirely without foundation and contradicted by the fact that the mesio-distal dimension of the second milk molar is larger than that of the second premolar.

In the odontological collection of the Anatomical Museum of the University of Amsterdam, there are about sixty preparations with a persisting second milk molar. Some general morphological remarks may precede our consideration of the evolu-

tionary significance of this anomaly. Among my material there are thirty natural preparations, the others being models. The anomaly occurs much more frequently in the mandible than in the upper jaw, the difference being so considerable that one may even maintain that in the latter it is a variation of great rarity. This difference between the jaws is not very surprising. As a rule a given variety is more frequent in one than in both jaws. However, there is a system in these differences. As to the absence of the third molar practically the same difference may be noted regarding the second milk molar. As mentioned in the second paragraph, the third molar is absent more frequently in the lower jaw than in the upper. On the other hand a distomolar—i.e., a so-called fourth molar—appears in the upper jaw more frequently than in the mandible. There is, I believe, a very remarkable relation between the frequency of these three anomalies in the two jaws. A distomolar in man is, as I have demonstrated in the first section, a reversion, being the reappearance of the third molar of the dentition of the platyrrhine monkeys; on the contrary the reduction of the third molar, and also the persistence of the second milk molar, are progressive variations. And so the conclusion is justified that reversion or regressive variation is more frequent in the upper jaw of man, whereas in the lower jaw the anomalies of a progressive nature preponderate. Therefore, in the way of modifying human dentition, the lower set averages to slightly precede the upper one.

If circumstances are favorable, the second milk molar remains in the jaw until the general shedding of the teeth begins, in consequence of senility. I recall the case of a colleague in which the second lower milk molars fell out after the 65th year. Furthermore it is very remarkable, that this milk tooth may still stand firmly in the jaw, even after the first and second molars have fallen out. This is demonstrated very clearly by figure 22, showing the left side of a mandible of about 35 years, in which m_2 still persists, in quite a sound state, while M_1 has fallen out and M_2 shows a deeply penetrating defect caused by caries. A case represented in figure 22 shows, at first sight, that a persisting m_2 in man cannot be interpreted as a patho-

logical appearance, caused by some irregular arrangement of the teeth, or by some obstacle in the normal substitution of the milk tooth; for if such were the case, the second milk molar of figure 22 surely would have dropped out, as, owing to the gap immediately back of it, it is in a very unfavorable position to maintain itself.

In carefully examining all specimens of persisting m_2 , present in my collection, I was not able to find a single instance in which the dental arch was an irregular one. In all cases the persisting milk molar participates in the structure of the arch as a perfectly normal element. One of my preparations is represented in figure 23. I chose this specimen because it is the mandible of a fairly old man, as is shown by the much worn occlusal surfaces of the incisors and molars.

As a last general remark I will draw attention to the fact that a persisting m_2 appears more frequently on the right than on the left side. This fact is not in accordance with the rule that in the left half of the human body anomalies seem to be more abundant than in the right one. So far as known to me this curious fact is as yet unexplained.

After these general observations regarding the persisting second milk molar, we enter into the problem of its significance from an evolutionary standpoint. I recall that stress has been laid upon the fact, that this anomaly is not at all of a pathological character, but that it must be regarded as a normal symptom of the future development of our denture. It is a variation of a progressive nature. Furthermore, emphasis has been placed upon the fact that this behavior of the second milk molar must be considered, together with the reduction of our third molar, from a common point of view.

The relation between the two variations is, I believe, a very simple one. The third molar of man is an element progressing toward reduction. It may be taken for granted that the human race will eventually be different from all other Primates by the complete loss of this component of the denture. But in consequence of this reduction the grinding surface is shortened; and it seems to me that at the anterior end of the molar region the

denture strives to compensate for the loss at the posterior end, for the grinding surface of the second milk molar is a larger one than that of the third premolar.

In thus interpreting these phenomena, there appears a very remarkable continuity in the developmental history of the primate dentition; for, in the first section of this communication, it is demonstrated that the dentition of the catarrhine Primates—including man—evolved from a platyrrhine monkey's dentition by the reduction of the hindmost molar, and the permanency of the third milk molar. And we now see that the dental variations, when considered in relation to each other, furnish a clear indication that an identical process is taking place in man today. This concordance leads to the conclusion that the future human race will differ from the present with regard to its dentition, in the same manner in which the catarrhine monkeys once differed from the platyrrhine.

This conclusion may be expressed in a clear manner, by the following series of dental formulas. In the first the dental formula of one of the Cebidae is written, in the second that of one of the Hapalidae, in the third the complete normal dental formula of man in its actual state, in the fourth the formula is given of a human dentition in which the third molar is reduced, and is indicated as a first distomolar, while the original first distomolar becomes the second; and in the fifth formula the final stage of the developmental history of human dentition is represented. In this formula the second incisor is also indicated as a lost element. In all these schemes the regressed teeth are placed in brackets, while the milk teeth are indicated in small print.

I Cebidae

$$\begin{array}{c} i_1 \ i_2 \ c \ m_1 \ m_2 \ m_3 \\ I_1 \ I_2 \ C \ P_1 \ P_2 \ P_3 \ M_1 \ M_2 \ M_3 \end{array}$$

II Hapalidae

$$\begin{array}{c} i_1 \ i_2 \ c \ m_1 \ m_2 \ m_3 \\ I_1 \ I_2 \ C \ P_1 \ P_2 \ P_3 \ M_1 \ M_2 \ [Di] \end{array}$$

III Homo: Normal state

$$\begin{array}{l} i_1 i_2 c m_1 m_2 M_1 [Pa_I] [Pa_{II}] \\ I_1 I_2 C P_1 P_2 [P_3] M_2 M_3 [Di] \end{array}$$

IV Homo: First phase of reduction

$$\begin{array}{l} i_1 i_2 c m_1 m_2 M_1 [Pa_I] [Pa_{II}] \\ I_1 I_2 C P_1 P_2 [P_3] M_2 [Di_I] [Di_{II}] \end{array}$$

V Homo: Second phase of reduction. Final state

$$\begin{array}{l} i_1 i_2 c m_1 M_1 M_2 [Pa_I] [Pa_{II}] \\ I_1 [I_2] C P_1 [P_2] [P_3] M_3 [Di_I] [Di_{II}] \end{array}$$

A brief synopsis of all transformations and reductions in the developmental history of the dentition of Primates is given in the three following simplified formulas, the first dealing with the dentition of one of the Cebidae, the second the dentition of man in its actual state and the third that of the future construction of human dentition after my conception. The first of these runs as follows:

$$I. I. C. P. P. P. M. M. M.$$

the second

$$I. I. C. P. P. M. M. M.$$

the third

$$I. C. P. M. M. M.$$

After this summarizing account of my views as to the direction in which the evolution of human dentition is progressing, I now present arguments supporting the probability of the accuracy of this conception. The fundamental point in it is the relationship maintained between the reduction of the hindmost molar and the evolution of the second milk molar. Our first task is to furnish the necessary evidences of this latter evolution.

As already mentioned the common view with regard to a persisting milk molar is that from some cause or other this tooth could not be shed, either because of lack of space, or an anomalous position of the second premolar in the jaw. This explanation is entirely erroneous. I do not pretend that in other

cases, and especially in the canine, the persistence of a milk tooth may not be caused in this manner, but this explanation is undoubtedly an incorrect one regarding the second milk molar. I have been able to prove this in a decisive manner by radiographically examining thirty jaws with a persisting milk molar. A radiographical photograph was made of each of these objects. What appeared from this examination? In only two cases was the second premolar present in the jaw, lodged in a horizontal direction beneath the roots of the second milk molar and first molar. Only these cases are really pathological, as the others, showing not the least trace of a second premolar, cannot be considered as such. My material included eight jaws in which the persisting milk molar occurred on both sides. I have examined thirty-eight cases of persisting milk molars. From this investigation it is proved that such an occurrence cannot be due to an irregularity in the topography of the second premolar, because this tooth is absent.

Figures 24 and 25 represent two of my preparations. Both cases show jaws of fairly aged individuals, as indicated by the worn occlusal surfaces of the molars.

These figures show, in the first place, that the persisting milk molars participate in an entirely regular manner in the construction of the set, as well from the functional as the morphological aspect. It is an important fact that the occlusal surface of the milk molar is on the same level as that of the preceding premolar and the succeeding molar, for there is a perfectly regular occlusion of the upper and lower set. Furthermore figures 24 and 25 establish the fact, that the persisting of the milk molar is attended with an absolute absence of the second premolar. Notwithstanding the most careful examination of both radiograms it is impossible to discover even the smallest trace of this tooth, and the same is true of all other cases. This is a fact of great importance, for it proves that there is an inborn functional correlation between the second milk molar and the second premolar. The loss of the latter tooth and its substitution by the first, are not two independent events, but one indivisible process. Because the individual, in consequence of heredi-



24



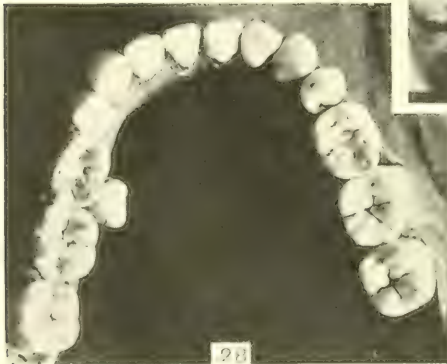
25



26



27



28

tary factors, was of a progressive nature with regard to the development of his dentition, his dental lamina produced a second milk molar able to function during a period of life much longer than normal, and to accomplish this the appearance of the germ of a second premolar was suppressed.

Perhaps, among the readers of this article, there is some one whose opinion differs as to the relation between the absence of P_2 and the persisting of m_2 , and who inclines to a more mechanical explanation. From this standpoint the absence of the germ of P_2 , should be considered the primary event and the persisting of m_2 only the necessary consequence of it, because a succeeding tooth, pushing out the milk tooth, is lacking. I cannot concur with this opinion. I think the persisting of m_2 and the absence of P_2 are due to a common biological, aetiological factor, this factor being the tendency of our dentition to substitute the second milk molar for the second premolar. This factor exerts a simultaneous influence on the dental germ of the second milk molar and that of the second premolar, stimulating the first and suppressing the development of the latter. From this standpoint the persistence of the milk molar is not a secondary consequence, but an occurrence as primary as the absence of the second premolar. But I admit that the relationship between the two occurrences may be viewed also from the mere mechanical standpoint. But this is not my view.

Figures 24 and 25 show a point of difference. In the first figure the individual possessed, with his persisting milk molar, the three normal molars also. And this person, during his adult life, was actually in possession of four molars in the lower jaw. The individual from whom the photograph in figure 25 was taken, was on a higher level of evolution, for in this case there occur two progressive variations, viz.: persisting of the second milk molar and absence of the third molar.

Although exceptional in people of today, such cases will be the normal condition in the human race of future ages.

It may be remarked, that the coincidence of the two variations in the case of figure 25 is a merely casual one. That this is not the case can be demonstrated in the following manner.

I estimate that a persisting milk molar occurs once in about 400 individuals, i.e., in 0.25 per cent. As mentioned above, according to de Terra the absence of the third milk molar occurs in nearly 12 per cent. This agrees with my examination of the voluminous material at my disposal, in which I found an absence of this tooth in about 14 per cent. Therefore it is a very minute chance that both anomalies, if wholly independent of each other, occur simultaneously in the same individual. Regarding the occurrence of both variations in one individual, the investigation of my collection has brought to light the following facts. Among thirty cases of a persisting milk molar, there were fourteen, or nearly one-half, in which the third molar was wanting. And when we consider that the latter generally is absent in only 12 or 14 per cent, the fact that it occurs in 50 per cent of the jaws in which the milk molar persists, is a convincing proof that there exists some relation between the two. This relation is also a strong argument in favor of my opinion that man is on the way to lose his hindmost molar, but at the same time a process of compensation is commencing at the anterior end of his molar region, in consequence of which the second milk molar is substituted for the second premolar.

Following above we shall consider the dentition as a whole.

It seems to me that there is some difference between the modes of progress in the lower and upper sets. In the lower set the third molar diminishes by degrees until the tooth is not developed at all. There is, therefore, not a gradual diminution in the size of the tooth, from a well developed strong object to a scarcely visible form, but the least developed third molar is still invariably of a fairly notable size. Therefore in the process of the disappearance of the third lower molar two phases are distinguished: in the first phase the object decreases regularly to a lower limit of existence, suddenly becoming absent. The second premolar of the lower set shows a somewhat identical behavior, this tooth either being present in a fully developed state, or wholly absent and the second milk molar substituted for it. This tooth is not subject to a gradual reduction in size. Therefore the character of the changes taking place in the lower jaw,

is that of a sudden disappearance of the elements. Between the total absence and the normal development of the elements a regular continuity of intermediate forms does not exist. And it is peculiar that one looks in vain in the lower set for instances of retrogression of the second premolar, as tests of the current process of disappearance of this tooth from our dentition.

In the upper set, on the contrary, the process of progression is much more regular. Here we may meet with a third molar reduced to a simple form. Here we may also see the second incisor regressing step by step to a very simple styliform element, and finally the second premolar also shows, in the upper set, degrees of reduction for which we look in vain in the lower set. Therefore the comparison between the two jaws, illustrates the accuracy of the statement made by Bateson that the minimum size of a tooth is different for different teeth. By this means the evolution toward the future type of our dentition proceeds in the upper jaw more slowly than in the lower. And this marked characteristic enables us to show more clearly, by means of intermediate forms, the direction in which this process of evolution is moving. This is demonstrated by figures 26 and 27.

In the foregoing pages I hope, I have demonstrated, that the future upper set of teeth of the human race will differ from its present construction by the loss of *a*, the second incisor, *b*, the second premolar (for which will be substituted the second milk molar), and *c*, the third molar. Now I beg to first consider the denture of figure 26. It is obvious that in this upper set of teeth the identical elements enumerated above are on the way to regression. The second incisor, the second premolar and the third molar are diminished in size equally on both sides. This specimen represents a very regular intermediate form between the normal dentition of the man of today and the future dentition of the human race. Now let us examine figure 27. This model, taken from a present day adult, fully represents the future dentition. The second incisor is lost, the development of the second premolar is suppressed and for this tooth is substituted the second milk molar, and finally the third molar is also absent. Functionally, this dentition, I frankly admit, is not of

a superior quality, but from a morphological and evolutionary standpoint this object is a very valuable one because it is the actual incorporation of all the theoretical and prophetic deductions at which we arrive in the foregoing paragraphs. Furthermore, the object does not at all impress one as a pathological one.

In the lower jaw analogous instances occur more frequently. I recall that cases in which the second milk molar persists and the third molar is absent, are not extraordinarily rare in the lower set. The reduction of the second incisor in the upper jaw is an indication of the occurrence of a *future denture* in this jaw more seldom than in the lower. In the former the reduction of three elements must concur, in the lower only two, hence the chance is greater in the latter than in the former.

Finally, I wish to call attention to a very fine specimen in my collection represented in figure 28. It is the lower jaw of an adult (but still young) woman. In the right half of the jaw the second milk molar persists and there are only two molars. As the radiographical examination brought to light, there was complete absence of the second premolar as well as the third molar on this side. This half of the denture represents, therefore, the normal progressive state of dentition described in the foregoing pages. The left half shows a very interesting peculiarity. The second milk molar persisted and the third molar was lacking on the right side, and in this respect this half was on the same level of progressive evolution as the right half. However, there was a difference. The second premolar, absent on the right side, was developed on the left, but obviously, from some cause or other, had not pushed out the second milk molar, and had erupted on the inner side of this tooth. It was actually standing opposite the dental interstice between the second milk molar and the first molar, thus illustrating the normal topographical relation between the elements of the first and second dentition.

A comparison of the conditions in the two halves of this denture leads to the view that the left half may be considered as an intermediate form between the normal condition and the

final progressive state, which has already been attained by the right half. In the latter there was no second premolar at all, the dental lamina obviously having lost the faculty to produce the germ from which this tooth should arise. On the left side, on the contrary, the dental lamina had still produced this germ, but it was not a strong one. Apparently it had undergone the influence of the evolutionary process, in consequence of which its developmental force was of a deficient quality. Hence the growth of the tooth was somewhat abnormal. Therefore the relation between the conditions in the two halves of the jaw is this, that on the right side of the jaw the progressive variation is complete, while on the left side the condition may be regarded as a less complete representation of the same variation.

Surely such cases are very misleading from a surgical point of view. For the practitioner, wishing to bring the whole denture into a regular and normal state, will extract the second milk molar on the left side, in order to bring the second premolar into its normal position. This treatment is fully justified, but there is a great chance that he may extract the second milk molar on the left side, when no premolar is lodged in the jaw, and by this treatment an irremediable gap is made in the arch, because, as mentioned above, the premolar is absent. This case indicates to practitioners that they must take the precaution never to extract a second milk molar before having proven the presence of the substituting premolar.

Before leaving this subject, I beg to compare figure 28 with figure 5. Apparently there is a similar variation in both preparations, three molars standing on the left side, and in both cases a premolar is erupted on the inner side of the arch at a point corresponding with the interstice between the first and second molar. But the morphological significance of the two premolars is different, for in the case of the *Macacus* of figure 5, it is the lost third premolar which reappears, and in the case of the human denture of figure 28, the second premolar reappears in a denture having the future construction. When in coming ages such a construction has become the normal condition, a variation such as occurs on the left side of figure 28 may be

judged as a reversion or atavistic variation. The morphologist of that time will consider such a variation as testifying to that past state of primate denture, when two premolars were normally present, in just the same manner, as in the case of figure 5 we may judge the additional tooth to be a reversion to the past state in which three premolars participated in the composition of the denture.

As a final remark I will call attention to the fact that a comparison of the conditions represented in figures 5 and 28, furnishes a strong argument in further support of my view that the first permanent molar of the catarrhine Primates, was originally the hindmost or third milk molar.

The sections of this communication treat principally of three problems, concerning respectively the past, the present and the future state of human dentition. And although the subjects of these sections are quite different, there is something common in the manner in which the problems are treated, and the standpoint from which they are viewed. This common standpoint is based on the preponderant value accorded, in the three sections, to the variations in our dentition. This point especially calls for remark, since some authors are determined to diminish the significance of dental anomalies, with regard to the study of the developmental history of our dentition. In the course of this article there has been occasion to point out that I cannot concur with this opinion. I believe, on the contrary, that just these dental anomalies furnish the most valuable data from which to build up the developmental history of this part of our organism. But it is necessary that we learn to interpret these data. In this paper I have shown how I have interpreted some of these. I hope my interpretations have been correct.

THE SINO-VENTRICULAR SYSTEM AS DEMONSTRATED BY THE INJECTION METHOD

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SIXTEEN FIGURES (FIVE PLATES)

The investigation described in the following pages was begun as a medical thesis. The first part of the investigation was merely a confirmation of work done by Lhamon '11 on the sheath of the sino-ventricular bundle. Since Lhamon's main conclusions were readily verified an extension of his work was undertaken, the results of which are here set forth.

It seems that the earliest record of any discovery of a muscular connection between the atria and ventricles of the heart, is that of Paladino in '76,¹ who, according to Retzer '04, is referred to by Bardeleben '76 as having found that "die Vorhofsmuskulatur endet nicht an den Annuli fibro-cartilaginosi, sondern geht grossen Theiles in die Ventrikelwand und die Papillarmuskel weiter." This rather indefinite statement had to serve for a description of the connecting link between the chambers of the heart until Gaskell '83 demonstrated that, in cold blooded animals, the auricular musculature is connected with the sinus musculature on the one hand and with the ventricle on the other. Since this connection was less easily demonstrated in the mammalian heart ten years elapsed after Gaskell's work before the muscular connection between the atria and ventricles of the heart of warm blooded animals was found.

Kent '93 found a definite muscular connection between atrium and ventricle in new born rats. This connection gradually became less evident in rats of greater age and in the adult Kent found only a few muscle strands. In the same year His, Jr.,

¹ Through Professor Meyer I am enabled to call attention to the statement of Prof. Giovanni Paladino in the *Anatomischer Anzeiger*, Band 46, 1914 "Ancora per una questione di priorità a proposito del fascio atrio-ventricularare del cuore."

definitely located the connection in mammals, describing it as having its origin from the posterior wall of the right atrium and extending to the ventricular septum and there dividing into a right and left branch. In '04 Retzer confirmed and extended this. In '06 the work of His was again entirely confirmed and extended by Tawara who succeeded in tracing the two branches down either side of the ventricular septum and demonstrated how the main branches divided into smaller branches and finally continued into the Purkinje fibres. Since '06 Tawara's work has been confirmed and more or less extended by many observers; notably, Keith and Flack '07, Fahr '09, Mönckeberg '08, Retzer, DeWitt '09 and Lhamon '11.

Although the attention of these observers was directed mainly to the course and distribution of the connecting link, there is nevertheless ample reference in their work to the constant presence of a connective tissue sheath around the bundle. DeWitt in making reconstructions from dissected specimens, found that she could not remove the sheath without breaking the inclosed strands. Curran probably saw the sheath when he succeeded in inflating a part of it with a blow pipe, but he made the mistake of confusing it with a synovial bursa which he described. The fullest investigation upon the sheath is that done by Lhamon '12, to which reference will be made later. Lhamon's injections were also subsequently confirmed by Cohn and Oppenheimer '11 and by Cohn '13.²

At this point a word concerning the nomenclature of the bundle is not out of place especially since it has already acquired no less than four different names. Before the time of His, Jr. no definite name was applied to it. The followers of Gaskell

² Although Dr. Lhamon's paper did not appear till March, 1912, his work was finished and his completed manuscript in my possession by July 22, 1911. He left for the Philippines, where he had accepted a professorial position, in beginning of August, 1911. The manuscript as published was sent to this Journal and as shown by the official stamp was accepted for publication on Nov. 3, 1911. Hence Lhamon's priority which has since been privately acknowledged by Dr. Cohn and inferentially also by Dr. Oppenheimer as shown in my statement entitled 'Injections of the bundle of His' is beyond question. See Science, N. S., vol. 42, November, 1915—A. W. MEYER.

and even Kent after he had demonstrated it in mammals, merely spoke of it as the muscular connection between the auricles and ventricles. No specific name was applied to the system at this time because of the vagueness of the knowledge regarding its size, shape, structure and location. However, after His showed the location and the appearance of the bundle it became necessary and proper that the newly-described structure should have a definite name. Hering '05 was the first man to use the term, 'The bundle of His,' in honor of its discoverer in mammals. Hering who described the functions of the bundle says in one of his footnotes "His nennt in seiner 1893 erschienen Mittheilung (Arbeiten aus der medicinischen Klinik zu Leipzig) das den Vorhof mit der kammer verbindende Bündel das Uebergangsbündel. Da His dieses Bündel gefunden hat, schlage ich vor, es mit seinem namen zu verbinden und es das, His'sche Uebergangsbündel zu nennen." A great many workers wishing to establish a more descriptive term in accordance with the *B.N.A.* have employed the newer terms: the atrio-ventricular (auriculo-ventricular) bundle or the sino-ventricular bundle. Retzer suggested the latter very acceptable term. In his earliest work on the subject Retzer ('04) describes the bundle as "das Verbindungsbündel," or "das atrioventricular Muskelbündel," or simply the 'Atrioventricularbündel.' In Retzer's ('08) more recent work on the development of the musculature of the heart, he shows that the bundle arises from the sinus and not from the atrium. Because of this fact Retzer suggested the name sino-ventricular instead of atrioventricular bundle. The term 'Reizleitungssystem,' used so extensively throughout German literature, was first used by Aschoff and Tawara. Since the greater amount of evidence now points to the fact that the sinus bundle is a continuation of the sinus musculature, i.e., that there exists an unbroken strand of modified muscle tissue from the sino-auricular node (Keith and Flack) to the termination of the Purkinje fibres, it seems best to use the term, sino-ventricular system. It will be designated by the letters S.V.S. throughout the remainder of this article.

The most suitable way of studying the relation of loose, elastic sheaths is by the injection method. The application of this

method to the sheath of the S.V.S. according to Lhamon occurred to Dr. Meyer, at whose suggestion Lhamon, working on beef hearts, in 1912 easily succeeded in demonstrating the S.V.S. in situ from the main trunk to many of its terminal branches. Lhamon's excellent injections supplied us with a new and reliable means of study. By it one is enabled to get a good idea of the shape and ramifications as well as a lasting impression of the exact position of the system at least in the hearts of *Bos taurus*, *Ovis arises* and *Sus domestica*. For although models made by reconstructions and dissections have given a good idea of the shape and main ramifications they fail to give one a comprehensive view of the bundle in situ in the heart. A reference to Lhamon's figure and those accompanying this article will quickly make this evident.

The presence of a definite sheath around the S.V.S. is in perfect harmony with what we know of the other organs of the body. The muscles, bones and nerves have their sheaths, and the kidneys and spleen also have connective tissue capsules all of which serve as dividing lines keeping the parenchyma of the enclosed organs from coming in direct contact with the tissues of adjacent organs. Indeed, there are no naked or uncovered organs in the body.

Sheaths in general are closely applied to the enclosed organ or more or less loosely attached. The nerve trunks have loose sheaths which may be distended by injection mass. Key and Retzius '76, for example, were able to apply the injection method using Richardson's solution, for demonstrating nerve sheaths. These observers obtained pictures similar in some respects, to those produced by injection of the sheath S.V.S.

This investigation was limited almost exclusively to beef hearts. Ninety hearts obtained from an abattoir in San Francisco, were used and although twelve to fourteen hours had usually elapsed before they reached the laboratory they proved to be entirely satisfactory for injection purposes. Although on a few occasions several hearts could not be utilized until after seventy-two or more hours had passed, yet it was found that they invariably gave as good if not better results with the injection

method than the fresher hearts. My experiences in this matter are in accordance with Buhm's work on the lymphatics for he often noticed that the injection succeeds better "*bei Leichen, die schon etwas angefault sind, als bei ganz frischen.*" As a matter of interest, hearts from other species were tested in a manner similar to the injections made on beef hearts. Two hearts from recently dead new born colts were tried but with negative results although hearts from dogs, cats, sheep, calves and swine were also tried with more or less satisfactory results. Fresh human hearts were not obtained.

The technique necessary for the injection of the sheath of S.V.S. consists of apparatus and procedures similar to that ordinarily used in the study of the lymphatic system. The injections were made at all times with an ordinary No. 1 Luer hypodermic syringe of 2 cubic centimeters capacity with the finest steel needle obtainable. The most convenient manner of holding the syringe for careful injections was found to be similar to that of holding a pipett, i.e., the barrel is held between the thumb and middle finger with the index finger on the head of the piston. This position gives one the best advantage in holding the syringe in place after the needle is inserted and pressure is brought to bear upon the piston. This detail is a matter of no little consequence since the slightest movement of the syringe after insertion of the needle may injure the sheath and cause extravasation. As injection media, suspensions of India ink, and Gerotas Prussian blue mass were used. For microscopic study of the S.V.S. with the injection mass in place, India ink proved to be the most satisfactory although Prussian blue was used more extensively for study of the distribution of the system. Gerota's Prussian blue injection mass was made up according to his original formula: Two grams of Prussian blue with three grams of turpentine were ground up in a porcelain mortar. The mixture was then diluted with 15 grams of ether and filtered through a fine cloth. This affords a mixture of low specific gravity which penetrates into the very finest spaces. The solution will also keep for months if put in an air-tight jar. For study of cell outlines within the sheath of the S.V.S., weak solutions

of silver nitrate were used—a solution (1–400) was found to be satisfactory. This solution was used only when fresh hearts were obtained. The injected area was exposed to strong light for a few minutes until it assumed a brown color before it was examined.

The verification of Lhamon's work was found to be an easy matter. The first series of 12 hearts were used for this purpose and also to obtain a more practical knowledge of the technique necessary for the work in hand. The repetition of Lhamon's work was also facilitated by the presence of two of his injected specimens in the laboratory which were often referred to.

Within the left ventricle of a freshly-opened, uninjected beef heart one can see the distribution of the bundle in many places, for it appears under the endocardium as a pale, pink tracing against the darker myocardium. The main branch can always be seen passing down the middle of the septal wall. Having reached a point about half way between the apex and the aortic orifice it suddenly forks sending two or more large branches across the ventricular cavity to the posterior ventricular wall. Other branches remain in the septal wall and divide, unite and subdivide as they proceed towards the region of the apex. In this region numerous branches cross from the septal to the posterior ventricular wall which together with the above-mentioned larger bridges constitute the trabeculae tendinae, i.e., false tendons (pseudo-tendinous threads) as they are often called, of the left ventricle.

If a needle be properly inserted in the sheath of one of the fasciculi in any of the above-mentioned places in the region of the fine anastomosing fasciculi or the main branch, the surrounding system can be injected over a more or less extended territory. The direction in which the injection is made makes no difference so far as the flow of the injected fluid is concerned. The needle may be pointed up or down or transversely if anastomosing fasciculi are chosen which run in a transverse direction. It was found an easy matter to insert the needle in the sheath of any of the anastomosing fasciculi making up the network of Purkinje fibres on the ventricular walls.

The fasciculi of the S.V.S. are broader at their terminal branches than elsewhere in their course. The main branches are made up of a greater number of fasciculi of less diameter than those found in the terminal network. This fact makes the terminal network the favorite site of injection. By insertion of the needle within the main branch of the bundle it often happens that the point does not remain within the sheath of a single fasciculus but ruptures the sheaths of three or four surrounding fasciculi. Although the needle is not within the sheath of a single fasciculus the injected fluid may collect for a moment at the point of injection, being temporarily retained by the general loose fibro-elastic envelope of the entire bundle but finally seeking the points of least resistance the injection mass may enter the ruptured sheaths and follow the fasciculi more or less extensively through their entire distribution. Since the general sheath of the S.V.S. carries the blood supply of the bundle, it often happened that some of these vessels were injected as well as the fascicular sheaths. Since in the case of the flattened terminal fasciculi of Purkinje fibres, the needle could be inserted into each individual fascicular sheath the result was a well-defined injection with no extravasation into the surrounding vessels or into the myocardium.

Bearing these points in mind the complete injections were made by using some of the terminal branches of the system as a starting point and then gradually approaching the main trunk by a series of successive injections. After locating a suitable point to start from it was always an easy matter to make the succeeding injections since advantage could be taken of the distended sheaths produced by the first injection in the subsequent injections. In making more than one injection it was always necessary to clamp off the punctures made by the preceding one in order to retain the fluid in the injected spaces, otherwise the pressure from the second injection would force it out of the first perforations. In many of the hearts injected for study of the distribution of the S.V.S. in the left ventricle the left crus and many of its terminal branches were filled with injection mass from the right ventricle, i.e., the injection was made in the right

crus and forced up to the point of bifurcation of the two crura and then down the left crus into the left ventricle (fig. 1). It was found easy to fill the left crus in this manner but the complete network on the ventricular walls could not be filled without two to four more injections of about six cubic centimeters of injecting fluid made at different points on the wall of the left ventricle.

When the entire left portion of the S.V.S. was filled with the injection fluid its distribution was found to be much more extensive than can possibly be seen in a fresh heart or by dissections (fig. 1). By employing Gerota's Prussian blue injection mass even the very finest branches were made visible under the endocardium. As has often been mentioned in previous descriptions of the system, by far the greater part of it lies directly under the endocardium. This is particularly of advantage in using the injection method for the colored fluid which is distinctly visible through the translucent connective tissue sheath, and the endocardium affords a complete and accurate picture of the sub-endocardial distribution of the system.

As shown in figure 1, the distribution covers almost the entire ventricular walls. The main two trabeculae come across the left ventricle and are broken up into a network at the base of the papillary muscles. This network does not reach to the apices of the papillary muscles, however, but in nearly all cases stops at a point midway between apex and base. Passing around the papillary muscles the network extends under the cusps of the bicuspid valve approaching very close to the fibrous ring of the atrio-ventricular orifice. The network is not so complete on the septal wall. There is a region about one and a half or two centimeters in width around the base of the aortic orifice and another about one centimeter in width which follows either side of the crus as far as its point of branching, which contains no superficial network. On the posterior ventricular wall the network is found to be much more complete for only a single bare area, scarcely one square centimeter in size, can be found.

The left crus as it appears at its point of emergence from the sub-aortic musculature, is very flat and superficial and remains so until it reaches its point of branching where it sends branches

of a more oval and at times cylindrical shape, across the trabecular bridges connecting the septal and posterior ventricular walls.

The false trabeculae are numerous in the left ventricle and are found to be both short and long and of varying diameter. They have been thoroughly described in the human heart by Mönckeberg and Tawara, and DeWitt has also examined them microscopically in the beef hearts. DeWitt speaks of them as the pseudotendinous threads and found all of them to contain fasciculi of the S.V.S. with the exception of three or four fine threads which pass from the upper part of the septal wall across to their insertion in the apices of the papillary muscles. In her descriptions DeWitt follows the general scheme of Mönckeberg of dividing the threads into the following three classes. *A*, Threads having no cardiac muscle fibres at all but consisting of connective tissue only (*wirkliche abnorme Sehnenfäden*). Threads of this class were very scarce for only three or four existed in the left ventricle. *B*, Threads carrying both myocardial fibres and fibres of the S.V.B. The majority of the threads were found to fall under this head. *C*, Threads carrying only fibres of the S.V.B. with their connective tissue sheaths. Only two small threads were found in this class.

Particular attention was paid to the course of the injection mass to see if it passed through all these classes of fibres. In all cases it was seen to pass through the threads surrounding even the very finest, described by DeWitt. Subsequent histological examination in many cases confirmed the observed fact. The anastomoses of the fasciculi of the S.V.S. are especially pronounced within the terminal networks. Indeed, the only place where individual fasciculi could be dissected out for any distance was in the right and left branches. As shown in figure 6, a fasciculus was dissected out on either side of the left branch for a distance of one and a half centimeters.

The distribution of the right branch within the right ventricle is quite as complete as that found in the left. The manner of distribution is somewhat different however. The right branch passes down the right side of the septum to the septal attachment of the moderator band along which it passes to the base

of the anterior papillary muscle. Having reached the anterior ventricular wall, the right branch breaks up into the terminal network of the right ventricle (Purkinje fibres), similar to that found in the left ventricle. However, since the first point of branching is on the anterior wall it necessarily follows that in order to reach the septal wall the network must needs pass by way of the conus arteriosus, of the inferior and posterior junction of the anterior and septal walls and of the moderator band when this latter connection is of goodly size as is often the case (fig. 4).

Special attention was paid to the flow of the injection mass during its course along the right crus to see if any branches were given off between its point of origin from the crus commune and its point of branching at the base of the anterior papillary muscle. In no place along its course was a single branch given off. Curran mentioned a branch as being given off just as the right crus makes the turn passing along the moderator band. This branch he stated supplies the conus arteriosus, but DeWitt, in her dissections, was unable to demonstrate any branches passing from the right crus. False tendons are not so numerous as in the left ventricle. They all seemed to carry fasciculi of the system, however, even the finest threads being injected.

As mentioned above the left crus of the bundle branches on the left septal wall at a point half-way between the aortic orifice and the apex but in the case of the right crus no branching is found until the anterior wall of the right ventricle is reached. Hence there is a decided difference in the length of the two septal crura, the right varying somewhat according to the length of the moderator band while the left remains more constant. The right crus as measured from crus commune to the point of branching was found to average 7.5 cm., while the left crus measured in like manner averaged but 4 cm. The course of the right crus, especially from its point of origin to its turning point on the moderator band is signified by its decided oval or cylindrical form and its more deeply imbedded position as compared with the flatter and more superficial left crus (figs. 10-11). As it makes the turn necessary to cross the moderator band it flattens out somewhat thus causing the appearance of an

enlargement but it really is just a change from a narrow cylindrical to a flat broad form, the absolute size remaining the same in both cases and after the turn is made the branch assumes its original form.

One noteworthy feature concerning the distribution of the system within the conus is found in the manner in which the network terminates in the region of the pulmonary orifice. About three fourths of a centimeter below the fibrous ring of the orifice the injection suddenly stops around the entire circumference of the conus, thus forming a uniform boundary or limitation of the network. No reason could be ascertained why the termination should be in such uniform manner. It was found to be constant in all hearts injected. An investigation of the musculature failed to show any superficial intersection of fibres at this point or any other characteristics which might be responsible for this peculiar ending (fig. 5).

As in the case of the left ventricle the most satisfactory method of injecting the systems in the right is from the terminal network (Purkinje fibres) up towards the main branch. The network on the ventricular walls was easily filled and the injection mass then forced upward along the right crus and thence into the atrium. As mentioned above the right crus is somewhat embedded in the myocardium so that it is difficult to locate it until it has been filled with injection fluid. This is another good reason for starting the injection at a more superficial and lower level rather than from the right crus.

While injecting the right crus from below it was found that the fluid which had proceeded upward as far as the bifurcation of the right and left crura from the crus commune passed over into the left crus and followed it into the left ventricle. In attempts to inject the atrio-ventricular node (Knoten) only a small amount of the fluid would pass into it, because of the tendency of the greater amount to follow the less resistant path down the left branch into the left ventricle. In order to eliminate this difficulty and get more fluid into the Knoten, the following procedure was followed. The right crus was tied or clamped off below the point of injection and the left crus treated in a similar

manner at its point of appearance within the left ventricle. The only place to which the fluid could now pass is via the Knoten into the right atrium. In this manner as much fluid could be injected into the Knoten and under as great a pressure as the containing walls would stand. After making an injection it was found best to let the injected specimens stand for three or four days in alcohol or formalin in order to permit the injection fluid to adapt itself to its new quarters. Upon examination of such injected and hardened specimens, the atrial portion of the S.V.S. was found to be imbedded in the atrial musculature and connective tissue to such an extent that considerable dissecting was necessary before the true results of the injection could be ascertained.

On exposure of an injected Knoten the latter was found to have the shape so often described previously (fig. 4), i.e., the form of a large nerve ganglion not at all unlike the semilunar ganglion. Its position is very constant and it bears a definite relation to the surrounding structures. The main body of the Knoten rests upon the os cordis from which it is separated only by a thin layer of closely adherent connective tissue fibres. From the main body at least two main branches are given off. One branch extends from 5 to 8 mm. up in a superior direction and is apparently in direct connection with the sino-auricular (Kieth and Flack) node. A second main branch extends in the direction of the coronary sinus along which the injection could be followed for 7-10 mm and at times a smaller branch extends along the left extremity of the os cordis. Smaller inconstant branches along the inferior border of the body of the Knoten approximate the septal musculature, but none were found to pass beyond the fibrous ring. The crus commune in leaving the main body often passes through a sulcus in the os cordis before it divides into the left and right crura. This sulcus was found to be present in sixty per cent of the hearts examined. Its presence may be due to the bone developing around the crus commune or to the play of the crus upon the developing bone during the movement of the heart. In showing a cleaned os cordis to Dr. Meyer he called my attention to the decided smoothness of the sulcus

in contrast with the rough edges in other parts of the bone. However, since many of the bones develop into very irregular and abnormal shapes it is impossible to determine any constant relations of the Knoten and crus commune to it.

The character of the branching of the system throughout both ventricles was quite constant and characteristic in certain regions (fig. 7). The mode of branching may be classified under four heads: 1) A scroll-like branching especially pronounced on the anterior wall of the right ventricle where the right crus, in branching at the base of the anterior papillary muscle, sends a main branch up to the conus. This branch assumes a gentle curvelike course. 2) A very coarse network, the meshes of which are composed of numerous fine fasciculi or of single fasciculi which are very flat and wide (fig. 12). This form is always found on the right septal wall and is especially pronounced at the base of the posterior papilla. Intermingled with the coarse network there is always present an anastomoses of fine fasciculi which make up a finer network. 3) In the termination of the branching on the ventricular wall around the bicuspid and tricuspid orifices instead of a network or scroll there is found a termination of the branches into fine filaments. This is probably the most pronounced of the four types or arrangements and is generally free from intrusion of the other types. 4) A fine network made up almost entirely of single fasciculi. This type is especially evident in the apices of both ventricles but is found to a greater or less extent in all parts of the ventricles.

In many places one is not able to say which form of branching predominates since there exists an intermixture of all forms. Nor in any one place is it possible to get one type entirely exempt from all of the other three because of the gradual transition of one form into another. Nevertheless, the point to be emphasized is that there exist in certain places in the beef heart, certain predominating characteristics of branching. These characteristics of branching seem to be determined in all cases by the arrangement of the trabeculae carnae within the ventricle of the heart on which they lie. The different characters of trabeculae carnae in different parts of the heart are very constant and fall

into the four classes mentioned for the S.V.S. The branches of the S.V.S. follow the ridges of the trabeculae carnae and thus acquire a distribution similar in arrangement to them.

The general conception of the arrangement of the sheath of the S.V.S. is that it consists merely of an enveloping connective tissue layer which extends in among the fasciculi. As a matter of fact the sheath is more complicated than this. In each of the main branches which bear typical sheaths the following description holds. The fibres or cells of the S.V.S. are grouped into bundles, the fasciculi. Each fasciculus is surrounded by a definite sheath of connective tissue derived from the common enveloping sheath. The structure of the fascicular sheath is very constant and the degree of condensation remains the same in all the specimens examined. This cannot be said of the general sheath, however. This is made up of loose connective tissue even approaching areolar tissue in some portions, while in other parts a more or less condensed connective tissue is found. This sheath carries the blood vessels, nerves and lymph vessels. Because of the absence of any better term the name epifasciculum will be applied to the general enveloping sheath while the sheath surrounding the individual fasciculi will be designated as the perifasciculum. The perifascicular sheaths are in close contact with the enclosed fasciculi but are not firmly attached and may hence be distended by the injection mass.

The epifascicular sheath can be identified only on the main branches. In the terminal network the fasciculi are not bound together into bundles but in many cases run as single fasciculi. This fact accounts for the real fine networks seen in many places on the ventricular walls. Where several fasciculi are held close together they present a coarser network. Often single fasciculi shoot off from the bundles thus producing delicate fine interlacements very similar to delicate nerve plexuses. These terminal fasciculi, however, always retain their perifascicular sheaths which are as capable of holding the injection fluid as any found in the main branches.

Anyone studying the sheath of the S.V.S. cannot help but notice the similarity to the sheaths of nerve trunks. In the

case of nerve trunks the fibres are grouped into bundles, the funiculi, surrounded by a definite condensed connective tissue, the perineurium. The several funiculi, together with their sheaths, are still farther enveloped in a general fibro-elastic envelope, the epineurium, which extends among and is directly united with the perineural sheaths. The epineurium carries the blood and lymph of the nerves. The perineural sheaths are closely applied to the funiculi but are only attached by delicate trabeculae running across and terminating in the endoneurium. The spaces under the perineurial sheaths which have been injected by Key and Retzius ('76) with Richardson's solution, present pictures similar to the internal surface of the similarly injected perifascicular sheaths of the S.V.S. A great many more elastic than white fibres are present in the sheath thus permitting considerable stretching under a strong pressure. In testing the strength of the sheath it was found to stretch to such an extent that ridges were found on the endocardium before the sheath finally broke, permitting extravasation.

Noting the above similarities between nerve sheaths and the sheath of the S.V.S., it seemed probable that these sheaths might be further similar in having the same kind of lining cells. The cells lining the perineurial sheaths of nerves are flat plate-like endothelioid cells and resemble true endothelial cells in shape and reaction towards silver nitrate.

Numerous injections of silver nitrate solution into the perifascicular sheaths were made to determine the presence or absence of lining cells but all attempts to demonstrate lining cells were fruitless, nothing but a brown mass appearing under the microscope. However, after making further trials on real fresh hearts some unexpected results were obtained. Outlines of lining cells still failed to show, but in some of the preparations a reduction had taken place in the intercellular substance between the Purkinje cells. The outlines of these cells appeared as wavy lines forming a network, which is similar in appearance to the cell boundaries of endothelium and was at first mistaken for cells of the sheath. Not only was there found to be a reduction in the intercellular substance but the silver was also reduced by the

granular protoplasm of the Purkinje cells as shown in figure 16. Granules are especially pronounced around the periphery of the cells, and they are so closely packed that the outlines of the cells may be followed by merely following the dark band of granules. The dark wavy intercellular substance in many places coincides with the granular layer but is also found in many places where the granular outline is not shown. This is likely due to the inconstancy of the silver reaction. In one place the granules only are stained while in a third place both may be stained together. For demonstration of the intercellular substance of the Purkinje cells it is, of course, necessary to have real fresh hearts. Positive results were obtained only from warm hearts taken directly from the killed animal.

While injecting one of the preparations with silver nitrate it was found that the sheath had been ruptured while dissecting out the fasciculus, and that a small tag of Purkinje cells entirely devoid of a connective tissue sheath or covering was reflected on the slide. By teasing these cells under low power magnification with a needle it was found that they broke apart very easily. A few of them were transferred to a second slide and stained with Delafield's haematoxylin. Upon examination with the oil immersion they were found to be polygonal cells with two or more nuclei as shown in figure 14.³ A complete encircling striated border was always present around the periphery of the cells. These striae in many places seemed to cross to neighboring cells joining the striae of the latter, thus resembling the protoplasmic bridges found in epidermal cells. Many of the cells stained with silver nitrate were found to show cross striations similar to cardiac muscle fibres. The centers of the cells are devoid of striations but granules and irregular masses of sarcoplasm are stained by the silver nitrate. One or more nuclei were found in various places in each cell.

In a few cases the injection-mass was noticed to have entered clefts between the cells as shown in figure 13. The pressure of

³This conclusion harmonizes with that of Ranvier, who obtained similar results after maceration with "potash," but unfortunately Mr. King was unable to investigate this question more fully.—A. W. M.

the injected fluid may have helped to form these clefts yet it is interesting to note that the clefts always occur between the presumed cell boundaries and never extend into the cells. The weak points in the fasciculus seem to be in the intra-cellular region. Small canals entering the fasciculus were occasionally observed to be injected. Figure 13 shows such a small injected canal. They end blindly and seem to be similar to the clefts, i. e., inter-cellular in nature.

DeWitt regards the bundle as syncytial in structure. She bases her conclusion on: 1) the constant presence and continuation of the fibrils in the system, thus making the fibril the unit of the system. 2) the absence of any connective tissue penetrating the S.V.S. cells and 3) believes that the clefts mentioned by Tawara are caused by shrinkage during fixation. However, Tawara, in spite of the continuation of fibrils throughout the fasciculi, considers the system as being made up of independent cells for he noticed wavy lines which he believed to be the outlines of the cells and at times also observed clefts between the cells.

The results obtained incidentally by use of the injection method and by teasing seem to show beyond all doubt that in the beef heart the S.V.S. is made up of independent cells. The injection of the clefts with injection mass and the reduction of silver nitrate by the intercellular substance seems to offer confirmatory evidence in regard to this conclusion.

The technique used for microscopic study of the injected sheath and fasciculi was as follows: The endocardium was stripped from the injected region, thus leaving the injected fasciculi on the myocardium. A portion about one centimeter long was then cut out and laid face downwards on a slide. The myocardium was scraped off and nothing but the injected fasciculi and the intervening connective tissue and fat were left on the slide. A drop of glycerine and a cover glass were added and the preparation was ready for examination. This simple method affords a quick and, for some things, satisfactory method for studying the results of injection of any kind into the sheath.

By making microscopic examinations of fasciculi injected with Prussian blue it was interesting to note that extravasation into

the surrounding tissue was absent in all cases. Microscopical examination further showed that many of the fasciculi are very flat and wide and lie with the broad surface parallel to the endocardium so that a broad flat injected fasciculus looks to the naked eye as if extravasation must have occurred along its course (fig. 15). An increase in width at the point of uniting or branching of the fasciculi was also noticed. The intercellular substance was stained in many places by the Prussian blue as in the silver nitrate method.

Injectons into warm hearts with intravital methylene blue were also attempted for the purpose of demonstrating nerve endings or fibers within the sheath. The solution used was prepared according to Wilson's method. Since it was found that the sheath would not retain the solution and that all the surrounding territory was stained no satisfactory results were obtained although an insufficient number of trials, was made to justify any definite conclusions. It was noted in the sections examined that no fibers of any kind were found crossing the space distended by the injections. Nerves distributed to the bundle have been described but their fibers have been demonstrated only within the general epifascicular sheath (Wilson '09). No endings terminating on the cells of the bundle have been demonstrated. In order to supply the cells making up the fasciculi of the S.V.S. the nerve fibers would necessarily have to pierce the perifascicular sheaths and cross the intervening space but in none of the specimens examined was a single nerve fiber found crossing this space. It would seem from this that the S.V.S. receives its nervous connections from elsewhere than the nerves in the surrounding sheaths.

The above investigation was undertaken at the suggestion and under the supervision of Dr. Meyer whom it is a pleasure to thank for his assistance.

Since the completion of my manuscript in December, 1914 the very interesting article by Aagaard and Hall in *Anatomische Hefte*, Band 51, Heft 2, 1914 was brought to my attention. This number of the magazine did not reach our library until February 10, 1915 and came to my attention some weeks later.

The main points of difference in the results of the investigations of Aagaard and Hall and my own are that I found no difficulty in injecting the pigs heart or the right ventricle and Tawara's node in bovine hearts and that my injections are somewhat more complete. However, I was unfamiliar with the interesting historical aspects of the question revealed by Aagaard and Hall and did not succeed in injecting a few hearts of newborn colts.

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PLATE 1

EXPLANATION OF FIGURES

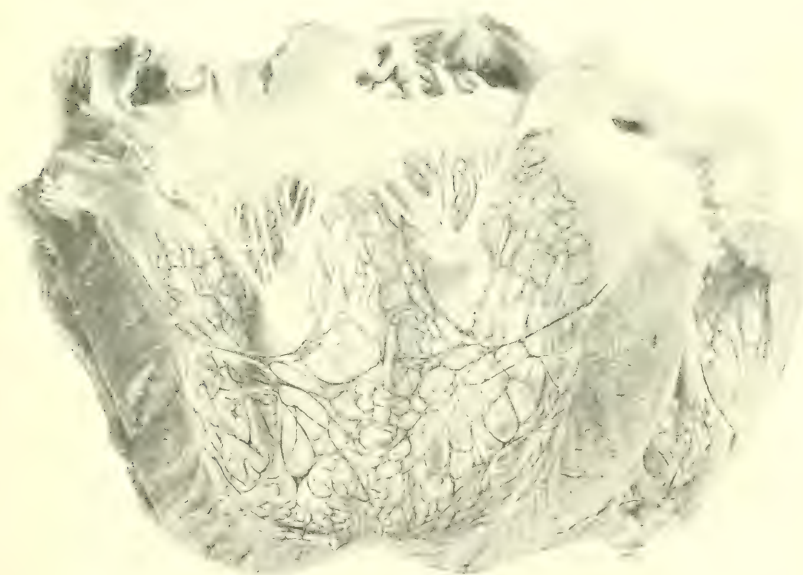
(Please view first four figures with reading glass)

1 Beef Heart No. 71. A retouched photograph showing the interior of the left ventricle. The injection shows the left branch of the bundle emerging from the subaortic musculature and branching into its terminal network over the walls of the ventricle. The left crus and the region of the apex extending up to the point of branching of the left crus and as far as the papillary muscles on either side was injected from the right crus within the right ventricle. The regions above the papillary muscles on the septal wall and under the cusps of the bi-cuspid valves were injected from the four points as marked in the figure by the small white rings.

2 Beef Heart No. 68. Interior of the left ventricle showing distribution of the S. V. S. around the papillary muscles and over the posterior ventricular wall. The ventricle was opened by an incision through the wall extending from the aortic orifice to the apex. Part of the right ventricle and moderator band is shown on the right. The left septal branch of the S. V. S. is divided longitudinally by the incision.



1



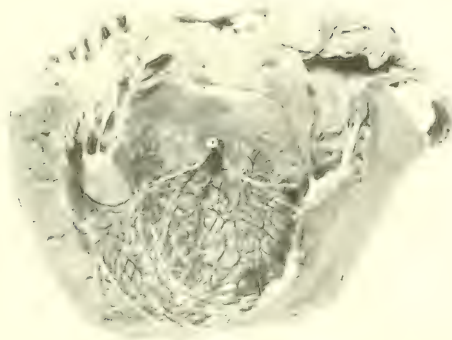
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PLATE 2

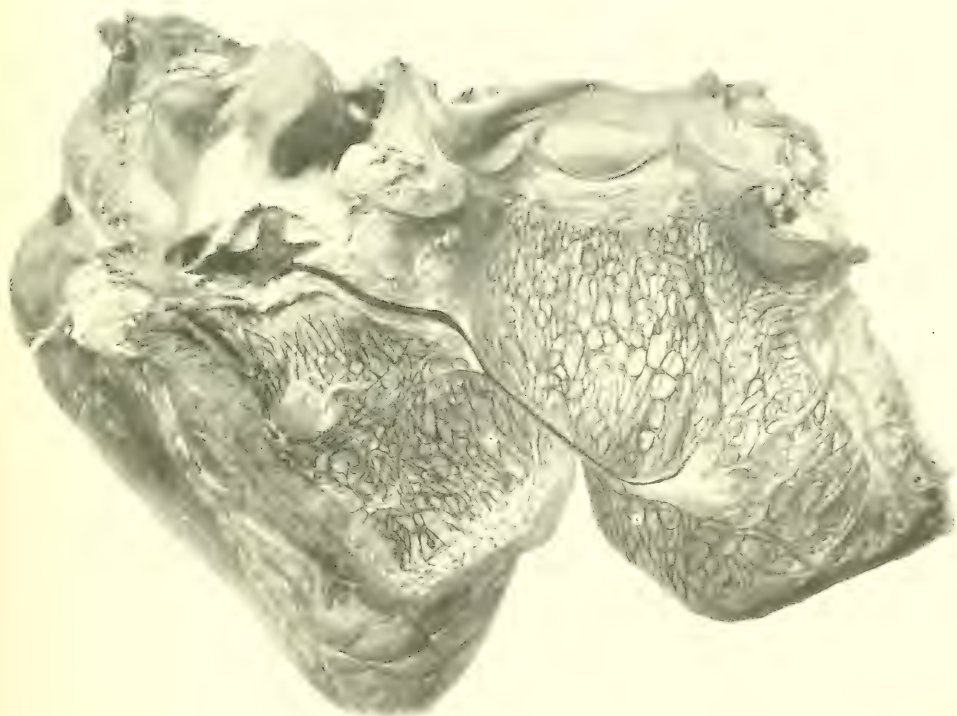
EXPLANATION OF FIGURES

3 Pig Heart No. 4. The left crus and a large part of the terminal network have been injected. (Purkinje fibers). The only point of injection used in this heart is indicated as before by the white ring near the left crus.

4 Beef Heart No. 55. The right ventricle, atrium and the conus are laid open. The Knoten, right crus and the terminating branches of the S. V. S. are all displayed by the injection mass. The Knoten and right crus have been dissected out of the myocardium and the sub-endocardial connective tissue. A large part of the endocardium has been stripped from the ventricular walls.



3



4

PLATE 3

EXPLANATION OF FIGURES

5 Heart No. 26. An inner view of the conus showing the termination of the network. *A.C.*, *R.P.C.*, and *L.P.C.*, anterior, right posterior and left posterior cusps of the pulmonary semilunar valve. *T.N.*, terminal network at the base of the pulmonary orifice ending in a comparatively straight line. *M.*, myocardium.

6 Entrance of the left crus into the left ventricle. The left crus has been dissected out of the sub-endocardial connective tissue and the epifascicular sheath so that only the fasciculi and their perifascicular sheaths are exposed. *F.*, a fasciculus dissected away on either side of the left crus for a distance of one and a half centimeters. *R.C.*, right coronary artery. *M.*, myocardium. *E.* reflected endocardium.

7 Different characteristics presented in the branching of the terminal network of the *S.V.S.* *C*, finer network found in nearly all parts. *D*, coarser network found around the borders of the atrio-ventricular orifices. *B*, scroll-like form found around the papillary muscle. *A*, coarsest network and irregular branching found principally on the right septal wall.

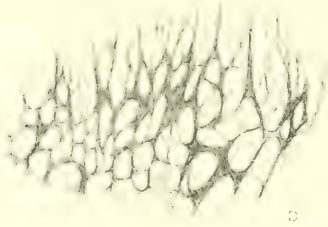
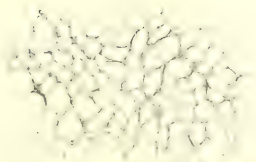
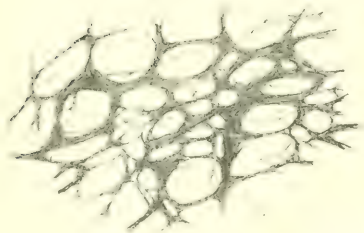
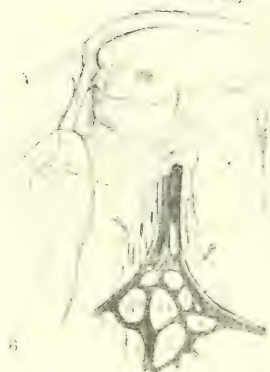
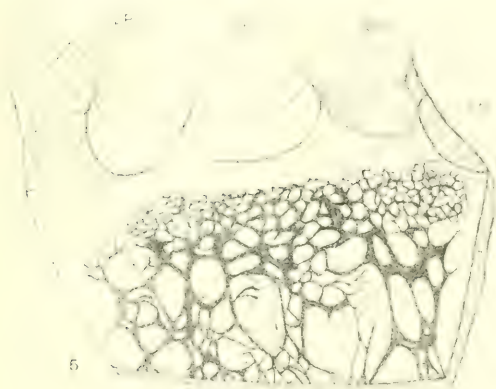


PLATE 4

EXPLANATION OF FIGURES

8 False tendon from the left ventricle of the sheep heart. *I*, injected mass *F*, fasciculus of the *S.V.S.* *E*, endocardium. *C*, connective tissue. Delafield's Haematoxylin and fuchsin stain.

9 Cross-section false tendon from left ventricle of pig heart. Treated in the same manner as figure 8.

10 Left crus; Cross section. *F*, fasciculi of the *S.V.S.* *E*, endocardium. *EP*, epifascicular sheath. *I*, injection mass. *B*, blood vessel. *M*, myocardium. *P*, perifascicular sheath. India ink injection. After Mallory's connective stain.

11 Right crus. *P*, an uninjected perifascicular sheath which is especially prominent due to shrinkage of the enclosed fasciculus during fixation. Treated in a similar manner to that shown in figure 10.

12 Cross section of a broad fasciculus taken from the apex of the left ventricle of *Bos taurus*. *C*, connective tissue of the epifascicular sheath. *P*, perifascicular sheath. *I*, injection mass. *F*, purkinje fasciculus. Mammory's connective tissue stain and injection with India ink.

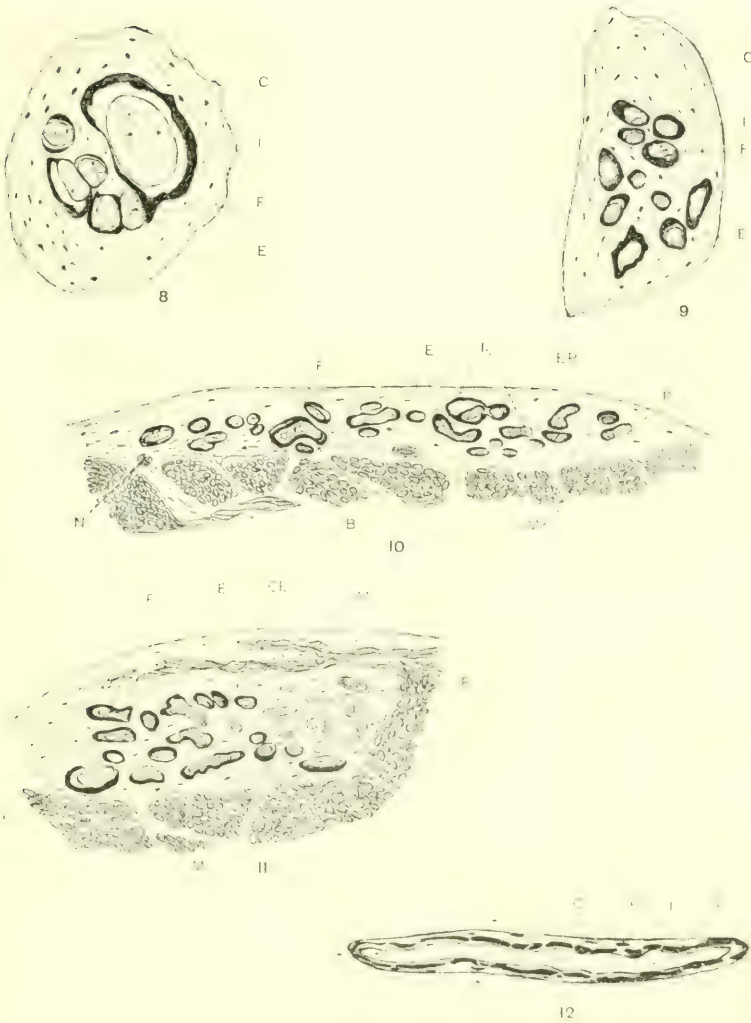


PLATE 5

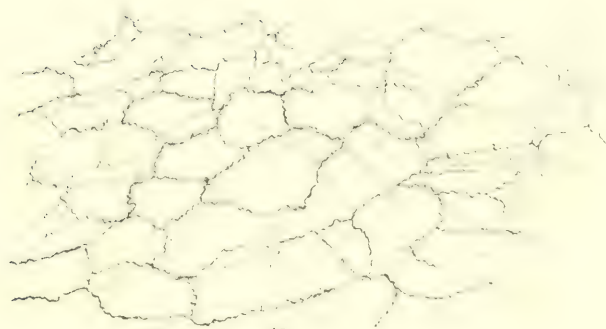
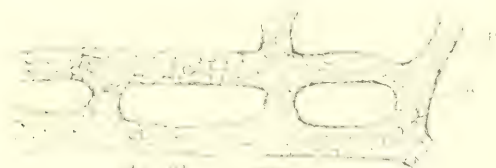
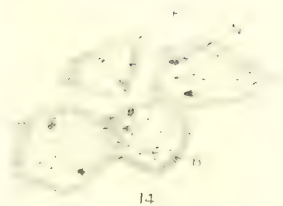
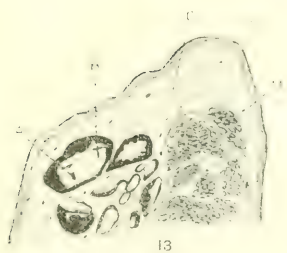
EXPLANATION OF FIGURES

13 Part of a false tendon from a beef heart. *A*, injected intercellular space. *B*, injected intercellular cleft. *C*, connective tissue. *M*, myocardium. Mallory's connective stain. India ink injection.

14 Purkinje cells from left ventricle. *F*, peripheral fibrils. *N*, nuclei. *B*, peripheral fibrils passing from one cell to the other. Delafield's Haematoxylin stain.

15 Flat view of a terminal fasciculus in left ventricle. *P*, perifascicular sheath. *A*, outlines of the Purkinje cells as shown by Prussian blue. *B*, large nodal point caused by the junction of several fasciculi. Prussian blue turpentine injections.

16 Flat surface view of a small part of a terminal fasciculus showing intercellular outlines as shown by the silver nitrate injection.



THE INTERRELATIONS OF THE MESONEPHROS, KIDNEY AND PLACENTA IN DIFFERENT CLASSES OF ANIMALS

JOHN LEWIS BREMER

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TWELVE FIGURES

In most anamnia the mesonephros begins its activity while the pronephros is still at its functional height. For a time both organs function together; then the pronephros undergoes degeneration and the mesonephros becomes the only functional excretory organ. This entire complex of processes has without further consideration been transferred to the relation between the mesonephros and the metanephros, and the question whether the mesonephros is actually functional in the amniota has never been seriously considered. Weber ('97) was the first to take it up and he endeavored to answer it in the following manner. He compared the time of degeneration of the mesonephros with that of the development of the metanephros; when he found that the mesonephros degenerated before the metanephros could exercise an excretory function, he has assumed that the mesonephros did not function, for if it had been active and had then degenerated before the metanephros had begun its activity, there must have been a certain period of development during which there was no excretion. Let us adopt this same method in considering the special case of the function of the human mesonephros. Already in an embryo of 19.4 mm. greatest length the majority of the mesonephric tubules are so far in process of degeneration that they cannot be regarded as having an excretory function. Of the 35 tubules of this embryo only four are actually still intact. In an embryo of 22 mm. greatest length none of the mesonephric tubules were capable of functioning; in all the tubulus secretorius had separated from the tubulus collectivus. If one inquires how far the development of the metanephros has progressed at this time, one finds that embryos of 22 mm. have just reached the anlage of the second generation of uriniferous tubules. The first generation, however, has as yet no fully formed Malpighian corpuscles. If, then, the mesonephros had functioned as an excretory organ, there must necessarily have been an interruption of this function on its degeneration. Consequently, I regard the question as to the functioning of the mesonephros as settled; it does not function as an excretory organ. This does not, of course, imply that it may not have been active in another manner unknown to us.

This paragraph by Felix in the Keibel-Mall Human Embryology¹ can hardly fail to arrest the attention of any one interested in the histological adaptation of cells and tissues to the physiological function which they perform. The similarity of the mesonephric glomeruli to those of the permanent kidney, of the convoluted tubules and the collecting tubules in both organs, the facts that developmentally the excreting portions of both organs are derived from the nephrogenic tissue and have practically the same history, and that both Wolffian duct and ureter open into the cloaca, all point so strongly to a similarity of the function of the two organs that it would need overwhelming proof to show that the product of the one gland is not at least very like that of the other, in other words to show that the mesonephros is not after all the 'middle kidney.' The statement is little short of iconoclastic, for ever since the time of Joh. Müller and von Baer the Wolffian body has been known as an excreting organ, continuing and gradually taking over the work of the pronephros, functional in adult life in lower animals, but replaced in turn in the higher orders by the permanent kidney. Apparent proof of their activity is given by Nicolas, who saw fine droplets of elaborated material in the epithelial cells and in the lumen of the tubules, and more especially by Bakounine, who after injections of sulphate of indigo into the aorta or into the vitelline vessels of chick embryos found in the epithelium of the Wolffian tubules the usual coloration given by this dye in the kidney.

A critical examination of Felix's argument shows that it is based on a single fact, namely, that the mesonephros in man degenerates and therefore ceases to function, before the kidney is capable of activity, thus leaving the embryo without an excretory organ during a part of its existence. If during a part of its existence excretion is not provided for and may therefore be assumed to be unnecessary, why should it have been necessary previously? If it is not necessary previously, then the mesonephros, apparently active previously, must have had some other, as yet unknown function.

¹ Vol. 2, p. 868.

Weber, by whose paper Felix was admittedly influenced, brings additional facts to bear on the case. He notes that in rodents the embryos have either a small and short-lasting mesonephros, as in the guinea pig and the mole, or small organs totally lacking in the essential glomeruli, the tubules ending in blind enlargements, as in the rat and the mouse. On looking for the receptacle for the mesonephric urine, presumably the allantois, he finds that it does not exist as an entodermal sac in some rodents, and is always a slender tubular reservoir in man. He reviews to a considerable extent the literature in regard to the opening of the urogenital sinus, seeking a possible channel for the urine into the amniotic cavity, and concludes, in spite of a number of observations by others, which he quotes and which place the date of this opening at various periods from 7.0 mm. to as late as 20.0 mm., that this channel is not open in man until the embryo has reached a length of 14.0 mm., whereas the mesonephros is apparently in full activity at 11.5 mm. "Sonach müssten wir uns zu der Annahme einer Sekretstauung in den ableitenden Wegen mit all' ihren Folgen verstehen, oder wir müssen, und das ist wohl zweifellos das richtigere, auf die Annahme der lebhaften absondernden Funktion verzichten."² This last argument is later discounted by Keibel, who says that though the presence of well developed and numerous mesonephric glomeruli does not show absolutely that the mesonephros secretes urine, yet even if a very small amount of secretion were present, it does not necessarily follow that the urogenital sinus must be open at this stage, thus indicating his belief in the ability of the human allantois, narrow as it is, to store a small amount of fluid. Weber also lays stress on the interval between the beginning of the progressive degeneration of the mesonephros and the development of the kidney to a stage where it can be considered functional, and accuses Nagel of disregarding this period in his statement that both organs, the provisional and the permanent kidney, are for a time active side by side. The figures of 22.0 mm. for the beginning of involution of the mesonephros, and of 30.0 mm. for the development of the first renal glomeruli in

² Weber, loc. cit., p. 67.

man are given, and the kidney could not be considered fully active till much later. Other authors place the beginning of the involution much earlier. Finally Weber refers to a report by Ahlfeld of a case of the birth at full term of a child with entire absence of both kidneys, and suggests that not only the mesonephros but even the kidney may be functionless for excretion in intra-uterine life. English also reports several cases of the obliteration or stenosis of the urinary passages in fetuses and in the new-born. Usually death occurred in the sixth to the eighth month, but in other cases, which were very surprising to him, the child was born healthy, and showed uraemic symptoms only after two or three days.

One stumbling block was found, however, by Weber in his studies, namely, the conditions existing in the pig. The pig differs absolutely from the other animals he examined by retaining an apparently fully functional Wolffian body up to the time when the kidney should be well able, from its anatomical development, to take over the work, leaving no gap when neither is available. But this exception where a continuous secretion is possible, but not proved, should not, in Weber's mind, vitiate his conclusions drawn from so many cases where a continuous excretion is impossible.

Had Weber gone further in his investigations he might have found more of these exceptions. Among mammals the cat, the sheep, the opossum, and in other classes the birds and reptiles all retain a functional mesonephros until the kidney is ready, the lizards using the provisional organ, according to Wiedersheim, sometimes for a year or more after hatching. Of the continuous activity of the urinary organs in the lower forms Felix seems to have had no doubts; his statements refer only to mammals and to the supposed error of assuming the sequence of events to be the same in mammals as in birds and reptiles. But if many mammals, and those of quite different orders, are found to show the possibility of a continuous urinary excretion, the status of the urinary organs is more and more established, and it is increasingly hard to believe that this continuous excretion is not universal.

The possibility that some other fetal organ might assume the excretory function during the interim when neither the mesonephros nor the kidney is apparently capable of activity, or might even in some animals like the rat and the mouse replace the Wolffian body partially or entirely is not considered by either Weber or Felix; yet that this is the case, and that this organ is the placenta seems to me to be strongly suggested by the facts brought out in this paper. Among physiologists the ability of the placenta to assume the rôle of the kidney is an accepted idea, derived from many experiments on the permeability of the placenta to a variety of substances passing from the fetus to the mother. Wertheimer, in Richet's *Dictionnaire de Physiologie*, published in 1904, mentions most of these experiments and concludes that "the activity of the kidney does not appear to be a function absolutely necessary during intra-uterine life; the excretory products which form in the fetal organism could be eliminated by the placental exchanges." Yet in this statement he was forced to overlook the contradictory evidence of certain physiologists, who had found that the placental permeability might not be the same in the earlier part of pregnancy as in the later days, and that, even in the same stages of intra-uterine life, one class of animals might differ from others in the results obtained. Thus Krukenberg found that materials which passed easily through the placentae of guinea pigs and rabbits were retained in dogs and cats. Since Krukenberg, comparative researches on the different classes of animals have not been undertaken, all experimenters relying with a singular unanimity on the results obtained from guinea pigs, rabbits, and man; but these exceptions to the general rules are very interesting, in the light of this present paper, as their explanation is simple, once the facts of the different methods of fetal excretion in the different animal classes are set forth.

It is my purpose first to show anatomically the presence in the placentae of certain animals of an excretory organ capable of serving the fetus when neither the Wolffian body nor the kidney is in activity, and second to point out the differences in respect to their excretory activity in the types of animals studied.

The evidence here adduced is wholly anatomical, based on the very similarity of tissues for similarity of function which Felix and Weber seem to deny. Moreover it is only the glomeruli of the mesonephros and kidney whose counterparts are recognized in the placenta. Further investigations will be necessary to distinguish, if indeed it is found possible to do so at all, the cells of the placenta which correspond to those lining the convoluted tubules of the excretory organs.

THE GLOMERULUS

The essential part of a glomerulus, whether of the mesonephros or of the kidney, is the arrangement of the inner capsule covering the knot of capillary blood vessels. The epithelial cells of this layer, when it is first recognizable, are of a rather tall cylindrical type, as we know from the researches of Stoerk, Huber, and others. With further development the cells become more flattened, but not to the shape of the usual squamous cells, such as those lining serous cavities, with the nucleus sharing in the flattening process, and no part of the cell much thicker than another. The modification consists in the formation of an extremely thin, flange-like process, extending usually from one side of the cell, while the remainder of the cell, including the nucleus, retains its cuboidal shape. At the same time the flanges or plates of adjacent cells seem to fuse so that cell limits can no longer be recognized. In some glomeruli, more commonly those of the mesonephros, the plates at first represent the bases of the cells, each nucleus with its surrounding protoplasm protruding beyond the general level (figs. 2 and 3); in other glomeruli the surface of the epithelial layer is smooth, and the base uneven. But the first type can easily be, and actually is, converted into the second by the pressure from within the glomerulus of the blood in the capillaries. For it is only in contact with or directly overlying the capillaries that the thin plates are found, while the nucleated portions dip deeper between the vessels, leaving a smooth outer surface. The future development of the glomerulus consists of an expansion of the plates, until the nucleated

cuboidal portions of the cells are far apart, of the lobulation of the glomerulus to increase its free surface, and of the apparent fusion of the plates with the underlying endothelium of the capillaries, which thus seem to project uncovered into the inter-capsular space (fig. 1).

Long ago Drasch investigated the glomeruli of the kidney and was able to distinguish two types, differing in size, lobulation, and position, and also, more interesting in the present study, differing in the kind of sheath (Hülle) which, by gentle shaking, he was able to detach from the knot of blood vessels. In one type of glomerulus this sheath contained nuclei, in the other none, or only a very few, in both the imprint of the capillaries was plainly visible. Von Ebner, in the 6th edition of Kölliker's *Gewebelehre*, offers, I think, the correct interpretation of these facts in supposing that in the one type of glomerulus the surface of the cuboidal parts of the epithelium had become transformed into an exoplasm similar in structure to the plates, and that this surface layer with the plates could be detached, leaving the nuclear portion connected with the capillaries. This would account for the non-nucleated sheath; the whole epithelial layer, plates and cuboidal portions detached together, would furnish the other picture. Drasch has established two facts of interest to us, first, that the blood vessels of the glomerulus are actually covered by thin plates, which can be separated from the endothelium by certain artificial means, though in sections it is usually impossible to distinguish more than a single layer, so closely are the two applied; and secondly, that these plates are of a highly differentiated protoplasm, non-granular and rather stiff, in that they hold their shape even after being removed from the capillaries about which they have been molded. The first of these facts is important as explaining a very natural mistake made by Duval in his description of the placenta of the rabbit; the second shows that these modified plates have apparently become inactive membranes, through which a purely physical osmosis may take place, but which themselves may be supposed to be physiologically inert. A physiological activity is

presumably present in the glomerulus, but limited to the thicker, still granular portions of the cells.

I have considered the glomerulus thus minutely, and offer a drawing of a portion of one in normal activity (fig. 1) because of the great lack of accurate descriptions and figures in the text-books. This is the more remarkable in contrast with the elaborate care with which the tubules almost uniformly are described.

The presence of thin plates of epithelium covering the capillary blood vessels is, then, the anatomical indication of that part of the excretory function which takes place in the glomeruli, either of the mesonephros or of the kidney. But it is not a sure sign that excretion is actually taking place at the spot where it is found, and for two reasons. The first is that diffusion or osmosis is dependent on the proper pressure on either side of the membrane, and on the proper chemical constitution of the fluid on the two sides; failing these two requisites the plates may be present but inactive. The second reason is of a different kind, namely that another physiological process, the exchange of oxygen for carbon dioxide and water, also takes place mainly through the medium of thin plates overlying capillaries. In the 'breathing epithelium' of the lungs and of the gills of the different types of vertebrates there is again found the modification of the epithelial layer, originally columnar, to a succession of thin plates, covering capillaries, and nucleated masses of protoplasm either projecting or imbedded in the meshes of the capillary network. Here again is a provision for both physical diffusion and physiological active secretion or excretion. As far as the simple diffusion is concerned, there is no physical law to prevent the passage of certain urinary constituents, oxygen, and carbon dioxide all at the same time, even in opposite directions, through the same membrane, if the conditions on the two sides of the membrane are favorable. In ordinary breathing this exchange in two directions is manifest. In other words, in seeking to establish the fact of a urinary excretion from the placenta by showing there the thin plates in their proper relation to the capillaries, it is necessary as far as possible to elimi-

nate the probability of their use for fetal respiration; but even if this cannot be done definitely, the possibility should be borne in mind that both processes, if each is considered a purely physical diffusion, might go on simultaneously through the same thin plates.

THE MESONEPHROS

I have long been interested in the relative size of the Wolffian body in different types of embryos, because of its influence on their future development; as I have pointed out in earlier papers it seems to govern the time of the connection of the rete cords and the testis cords, the position of the spermatie or ovarian arteries, and the development of the renal artery with the consequent differences in the range of anomalies to be expected in these vessels. In this regard the commoner embryos may be grouped as follows; pig and rabbit, large Wolffian bodies; sheep, medium size to small; cat, man, guinea pig, and opossum, small; mouse and rat, practically none at all. It was perhaps not an unnatural mistake to suppose that the larger the Wolffian body, the longer it would remain active, yet this is not at all the case. The pig, as pointed out by MacCallum, has an increasingly large mesonephros up to the 40.0 mm. stage, and no reduction in its size until 95.0 mm. The rabbit, on the other hand, while possessing at first almost as large an organ as the pig, begins to show mesonephric degeneration at about 20.0 mm. In the sheep, though the gland is never large, it is retained as in the pig, increasing up to about 25.0 mm., and with no reduction at 48.0 mm. It is for this reason that the sheep was classed among those animals with large Wolffian body in my study of the testis and rete cords; the presence in late embryonic life of this organ was erroneously thought to prove its great size in earlier periods. The opossum retains an active Wolffian body after birth, and it is not replaced by the kidney for a considerable time. This in itself would prove that for this class of mammals, as in the lizards, the mesonephros is certainly functional as a urinary organ. The cat, the guinea pig, and man all have small Wolffian bodies, yet in the cat they increase

steadily to 32.0 mm., while in the guinea pig and in man signs of involution soon set in, and by 15.0 mm. in the guinea pig the organ can no longer be considered active. In man the statements of many investigators are so conflicting as to the time of this involution that it is impossible to draw any very definite conclusions, but from my own observations it seems that the Wolffian body may be functional later than has been usually supposed, though many of its glomeruli certainly degenerate early.

In order to have some definite ideas as to the relative length of time during which the Wolffian body, or at least that part of it represented by the glomeruli, may be considered functional in the different kinds of mammals studied, I have counted and measured the active glomeruli in different ages of embryos. This can give at best but crude, inaccurate results, since the diameter of a glomerulus may have no definite relation to the area of its surface and to the area of the epithelial plates on that surface; the diameter tells us nothing of the amount of lobulation, each new lobule increasing the surface area, nor of the ratio of epithelial plates to the protoplasmic bodies of the epithelial cells. Still the inaccurate results are sufficiently striking for the purpose of showing the great variation which exists between the different types of animals.

At one end of the scale are the embryos of the mouse and rat, which never develop mesonephric glomeruli. Next to these come the embryos of the guinea pig, in which the glomeruli are immature at 8.0 mm., are never large or numerous, and have undergone marked degeneration at 15.0 mm., when there are only fourteen in one Wolffian body, with an average diameter of about 52 micra. Even these few small glomeruli could hardly have been properly functional, as they lack almost entirely the epithelial plates. The mole, according to Weber and others, is in the same class as the guinea pig, with few, short-lasting glomeruli. In the rabbit there is early a large organ; an embryo of 9.6 mm. has about forty active glomeruli on each side, with an average diameter of 110 micra. This increases only to forty-two glomeruli in an embryo of 14.5 mm., as many

of the anterior ones are already lost; but there is a marked increase in lobulation and in the size of the individual glomeruli, whose average diameter is now 185 micra. By 21.0 mm. the organ has begun to diminish, in that there are only thirty-four glomeruli on a side, with an average diameter of 200 micra; and soon after this all traces of the mesonephric glomeruli have vanished.

The mesonephros of man and its degeneration have been especially studied by Felix in the article in the Keibel-Mall Human Embryology before referred to. He shows a table of the number of mesonephric tubules found by him in many human embryos up to 21.0 mm. in length, and calls attention to the constant degeneration of the anterior tubules from 7.0 mm. on, and the addition of new tubules caudally. The number of tubules is not an absolute measure of the number of glomeruli, since some of the tubules may branch, or two tubules may lead from a single glomerulus; but it is sufficiently accurate for the present purposes, as the irregular tubules are always few. He finds an early degeneration of the organ, and then a period of rest. "From the stage of 21.0 mm. greatest length onwards, all embryos show a rather constant number of mesonephric tubules in the lumbar segments, but these tubules are almost all broken in one or several places." In the quotation heading this article he again states definitely that none of the mesonephric tubules in an embryo of 22.0 mm. greatest length were capable of functioning, as in all the tubulus secretorius had separated from the tubulus collectivus. In man, according to Felix, the rete tubules connect only with the tubuli collectivi, so that the secretory portion of all the tubules may disappear before this union takes place.

My own observations do not entirely agree with this account. In a former paper I have shown rete tubules connecting with the remains of the mesonephric corpuscles in certain cases, remains which are recognizable as late as the seventh month. This assures us that at least a few of the mesonephric tubules have remained intact (though of course not necessarily functional) from glomerulus to duct up to the time of the urogenital union.

In human embryos of 37.0 mm. to 40.0 mm. there are usually about a dozen mesonephric glomeruli on each side, some undergoing degeneration, others showing every sign of normal activity, with bulging epithelial plates over fully distended capillaries, and no change of the flat epithelial cells of Bowman's capsule to a cuboidal layer, which is one of the characteristic signs of degeneration, according to von Winiwarter. By reconstruction I have been able to follow the tubules from certain of these apparently normal glomeruli to the duct. In each case, at the junction of the tubulus collectivus with the tubulus secretorius, the lumen suddenly narrows and the epithelium becomes indistinct, stains lightly, and is obviously changed from the normal. In other tubules actual breaks occur at this spot, as Felix noted; but I think he has been misled by the fact of these actual breaks at degenerated portions of some tubules to the conclusion that in all the tubules there is a loss of continuous lumen. The lumen is present in some tubules, and these are probably the ones found acting as ductuli efferentes in the few cases where the rete joins the corpuscle, instead of the tubulus collectivus.

In man, then, there is a small Wolffian body, early developed to its full capacity, but retaining its function, as far as the glomeruli are concerned, only until the second or third month of intra-uterine life, when the embryo has reached a length of 20.0 mm. to 30.0 mm. In a 10.0 mm. embryo there are about thirty-four active glomeruli in one organ with an average diameter of 125 micra; at 13.6 mm. there are about the same number, each of about the same diameter, but greater efficiency is probable as each glomerulus is more deeply lobed. At 30.0 mm. there is still the same number. At 40.0 mm. the number of glomeruli is reduced to about a dozen, and some of these show signs of degeneration.

In sharp contrast, in this respect, to the embryos of mouse, rat, guinea pig, rabbit, and man are those of the pig, sheep, and cat. In the pig, at 8.0 mm., there are fifty-one glomeruli on one side, forty-five on the other, according to MacCallum; my figures are slightly higher, fifty-four active glomeruli on each

side. The average diameter, 200 micra, is very large when compared with those of the early embryos noted above. At 11.0 mm. there are sixty active glomeruli, at 24.0 mm. eighty on each side, and in each case the average diameter has reached 325 micra, six times that of the glomeruli of the guinea pig. At 95.0 mm. many active mesonephric glomeruli with the same large diameter are present, and in the same specimen the kidney also contains two or three rows of apparently fully active glomeruli, with bulging epithelial plates. It seems certain that in the pig, as pointed out by Weber, the activity of the mesonephros overlaps that of the metanephros, as is the case in birds, reptiles, and the opossum.

In the sheep the comparison of the size and number of the mesonephric glomeruli is an even less accurate guide to their relative activity than in the other mammals studied, because of the peculiar type of structure which the anterior corpuscles present. As I have recently shown,³ the first twenty or thirty corpuscles are without true glomeruli; the lower or caudal ones are of the usual type. But as the number of irregular, atypical corpuscles is approximately the same at all ages, their presence does not vitiate the count to any great extent. In a sheep embryo of 6.6 mm. there are six active glomeruli on each side in addition to the twenty or thirty atypical corpuscles, and their average diameter is 150 micra; at 15.8 mm. there are fifty normal glomeruli, of 230 micra; and at 40.4 mm. again fifty on each side, but with an increased diameter, 285 micra. In the 40.4 mm. embryo the kidney contains several active glomeruli with an average diameter of 100 micra.

The cat of 7.6 mm. has about twenty active glomeruli in each organ, diameter 150 micra; at 15.0 mm. the number has increased to twenty-six, and the diameter to 165 micra, but each glomerulus is much more lobed. At 39.0 mm. there are thirty glomeruli with an average diameter of 200 micra, and in this same specimen the innermost renal glomeruli are developing epithelial plates. At 85.0 mm. the Wolffian body has dis-

³ Bremer, loc. cit., p. 3.

appeared, but several rows of renal glomeruli are apparently active, as is shown in figure 4.

Similar figures for the chick may be interesting. At 7.5 mm. twenty active glomeruli are found in each Wolffian body, with many more in the earlier stages of development; the average diameter is 95 micra. At 15.0 mm. the number has increased to one hundred and eight glomeruli, and the diameter to 105 micra.

From these figures, grouped below in tabular form, two facts stand out as evident; first, that the different embryos can be classed as those which retain a functional Wolffian body until the kidney is ready to take up the work of excretion, and those

TABLE I

Number and diameter of active mesonephric glomeruli at different ages, in one Wolffian body

| | 6.6 TO 10 MM. | 11 TO 16 MM. | 21 TO 40 MM. | OLDER |
|-----------------|---------------------|---------------------|---------------------------------|---------------------------------|
| Rat..... | none | none | none | none |
| Guinea pig..... | none | 14, of 52 micra | none | none |
| Man..... | 34, of 125 micra | 34, of 125 micra | 12, of 125 micra | none |
| Rabbit..... | 40, of 110 micra | 42, of 185 micra | 34, of 200 micra | none |
| Cat..... | 20, of 150 micra | 26, of 165 micra | 30, of 200 micra | kidney |
| Sheep..... | 20+ 6, of 150 micra | 20+50, of 230 micra | 20+50, of 285 micra + kidney | kidney |
| Pig..... | 54, of 200 micra | 60, of 325 micra | 80, of 325 micra +kidney | many of 325 micra +kidney |

in which the Wolffian body disappears early, before the kidney has developed active glomeruli; and second, that within each of these classes, individual animals are provided with a very varying amount of excreting surface, showing presumably varying types of metabolism. In the cat, for instance, the paucity and small size of the mesonephric glomeruli up to the time when the kidney becomes active, indicate a greatly reduced urinary excretion as compared with that probable in the pig, with its enormous and numerous glomeruli. Another example of the difference in glomerular number and size in two animals which early lose their Wolffian bodies is found by comparing rabbit and man.

The total glomerular surface of each of the animals here studied seems to have a definite relation to the rapidity of its intra-uterine growth and the consequent length of the period of its gestation. The size of the animal at birth must naturally be taken into consideration, as no one would expect a large full term pig fetus, for instance, to have developed in as short a time as the new-born guinea pig. But in comparing the guinea pig and the rabbit, one may be surprised that the former, smaller animal should have a gestation period twice as long as the latter. The number of embryos can have no influence in this case, as the rabbit normally has a much larger litter than the guinea pig. There is obviously an actual difference in the growth rate, and this is correlated with a difference in the excretory surface in the embryo: the slower the growth rate, the less active the cell changes, and also the less rapid the formation of waste products to be eliminated. Other factors undoubtedly underlie the causes of the differences, and it is not intended to suggest that a small Wolffian body can cause slow growth, or vice versa, but a comparison of the periods of gestation, as given by Grosser, of the animals here studied with the table showing the size and number of their mesonephric glomeruli shows that, if the size of the animal be considered, the growth rate is evidently correlated with the glomerular surface. Grosser gives as the period of gestation for the rabbit, 28 days; for the rat, 35 days; for the guinea pig, 63 days; for the cat, 65 days; the pig, four months; the sheep, five months; man, nine months. The pig has a larger glomerular surface than the sheep, and a shorter period of gestation; man has less glomerular surface than either pig or sheep, and a longer intra-uterine life. If we consider the relative sizes of the cat and the sheep, the cat's period of gestation might be regarded as proportionately the longer of the two, perhaps comparable to that of man; and the total glomerular surface of the cat embryo is very similar to that of the human embryo. A systematic study of the comparative metabolism of different animals has not as yet been attempted, to my knowledge, though scattered facts such as the analyses of many types of urines are available. From the differences

found in the amount of excreting surface developed in these embryos and the correlated differences in their intra-uterine growth rates it seems probable that great variations exist, which, if persistent in the adult, should certainly be carefully considered in animal experimentation.

THE ALLANTOIS

If certain embryos excrete urine from the Wolffian body or kidney during their entire intra-uterine life, and others do not, there should be easily recognizable differences in the size of the receptacle for this urine in the two classes. In spite of the interest formerly taken in the time of the opening of the cloacal membrane, as a possible passage for the urine into the amniotic cavity, repeated chemical analyses of the amniotic fluid show that it may contain only traces of urinary matter, and thus make it extremely doubtful if this passage is ever used normally for any length of time. Failing this external outlet, the urine from both the Wolffian body and the kidney must pass to the allantois. In birds and reptiles the allantois is a large sac capable of containing a considerable quantity of fluid. The same is true of certain mammals. According to Grosser, Hertwig, and others, the allantois is an enormous vesicle in the pig and sheep; in the cat it is again of large size, though smaller than in the former two animals. In the rabbit, on the other hand, it "reaches no special expansion; it is here limited to the area of the placenta."⁴ In man and in the guinea pig, "that is, in those embryos which form no hollow allantois,"⁴ only the slender allantoic stalk is found; yet, as was mentioned above, this narrow cavity seems to Keibel capable of storing a small amount of fluid. Minot states that "the allantois in man increases very little in diameter after the second month,"⁵ the time when the Wolffian body, as we have seen, ceases to be functional. In the rat and mouse, even this small receptacle is absent, as an entodermal allantois never develops.

⁴ Hertwig, loc. cit., vol. 2, p. 250.

⁵ Minot, loc. cit., p. 355.

It will be seen at once that in the animals selected for study there is a close relationship between the size and duration of the Wolffian body and the size of the allantois. The cat has a smaller allantois than the pig or sheep, because its Wolffian body is less effective, but a larger one than the rabbit since the urine is accumulated throughout intra-uterine life in the cat, but only for a short period in the rabbit.

THE PLACENTA

The placentae of various mammals have been studied anatomically and physiologically by many of the best investigators, and the different types of placenta belonging to the different groups of mammals are well known. Grosser, one of the most recent writers on the subject, arranges the types as follows:

- A. Semiplacentae (placentae appositae)
 - a) semiplacenta diffusa. Type; pig.
 - b) semiplacenta multiplex. The ruminants.
- B. Placentae verae (conjugatae)
 - a) placenta zonaria. Type; most carnivora, cat.
 - b) placenta discoidalis.
 - 1. Rodents, rabbit, mouse, guinea pig.
 - 2. Insectivora.
 - 3. Cheiroptera.
 - 4. Primates.

In the apposed placenta, as its name implies, there is no definite union between the fetal and maternal tissues, no destruction of the maternal epithelium by the trophoderm of the chorion. The chorionic epithelium is apposed to the epithelium of the uterus, perhaps sending cell processes between the maternal cells, as maintained by Robinson, but separating from the maternal cells at the end of pregnancy without destruction of the uterine surface. There is no intimate relation between the maternal blood and the fetal vessels; nutrition and oxygenation of the embryo go on by means of the active absorption by the chorionic epithelial cells of products directly given to them by the maternal epithelium, or of the 'uterine milk,' the excre-

tion of the uterine glands and of the surface epithelium, with many maternal leucocytes added. Respiration and nutrition must be active secretory processes, as nowhere in these placentae is found any thin osmotic membrane in relation with the fetal capillaries. Granules of absorbed material have often been found in the fetal epithelium.

The conjugate placenta, on the other hand, is formed by a destruction of the maternal tissue by the trophoderm, a loss of the uterine surface epithelium and of more or less of the deeper layers, and the consequent pouring out of the maternal blood into the intervillous spaces. The chorion is bathed in slowly circulating maternal blood, and it is from this, instead of from the uterine milk, or by the transference from one epithelial cell to another, that the embryo obtains food material and oxygen. The maternal epithelium of the uterus plays no part; the seat of the transfer is the epithelial covering of the chorion and its prolongations, the chorionic villi.

An exception seems to exist in those rodents with the so-called 'inversion of the germ layers,' for in these the yolk-sac entoderm, after the loss of its distal layers and of the chorion originally covering it, is spread out over a portion of the inner surface of the uterus, and may receive nutriment from the secretion of the uterine glands. But this is at best an accessory source, as the placenta proper is in these cases also composed of chorionic prolongations, bathed in circulating maternal blood. Another exception is found in the 'green column' or border zone of the zonate placenta of the cat and other carnivora, a large reservoir of extravasated blood, between the uterus and the chorion, from which the chorionic epithelial cells, here of a tall cylindrical type, can be seen to ingest certain red blood corpuscles. This again is an accessory source of food supply, as it develops only in the second half of pregnancy, and has no noticeable effect on the size or activity of the true placenta.

It is the characteristic of conjugate placentae, then, that the fetal chorion and villi, of whatever shape they may be, are bathed in circulating maternal blood, and that the transference of material between mother and embryo takes place through the

fetal ectodermal epithelium. The character of this epithelium and its relations to the fetal capillaries, by which the material can be carried to or from the fetus itself, is of importance in this study, as it is through thin plates in this epithelium, closely covering the fetal capillaries, that osmosis of urinary matter would necessarily take place, if the present thesis is correct. Once passed from the fetal blood to the maternal circulation, these materials could be finally excreted by the maternal kidneys.

In certain placentae the extreme thinness of this epithelial layer and its close relation to the endothelium of the fetal capillaries are features of such prominence that they have been noted and figured by every writer on the subject. This is the case in the rodents. Duval, Minot, Grosser, all agree that in the placental labyrinth of these animals the earlier thick trophodermic layer becomes reduced to a very thin syncytium, with thicker areas containing nuclei, on one side of which is the maternal blood, on the other side the fetal capillaries (figs. 6, 7, and 8). In fact Duval⁶ goes further and reports the ultimate disappearance of the syncytial plates. The capillaries thus appeared to him naked in the maternal blood-stream. The other authors do not agree with this, and, I think, rightly; it is much more likely that the plates and the endothelium are so intimately associated as to appear a single layer, as in the renal glomeruli, where they have been proved by Drasch to retain their independence, as already stated above. The conception of the nucleated portions of the syncytium remaining in situ but isolated from each other is rather hard to grasp.

The rodent placenta is thus provided with a membrane presumably suitable for osmosis as well as thicker nucleated portions for active secretion or absorption. But the different types of rodents differ in the length of time necessary to attain this arrangement. In the rabbit this modification of the trophoderm, "*la période d'achèvement de l'ecto-placenta*," according to Duval, comes near the middle of pregnancy, at 25- to 30 days.

⁶ Duval, loc. cit., p. 119.

In the guinea pig, whose gestation is twice as long as that of the rabbit, it appears at about the same time, that is relatively early, the embryo in the same period having attained little more than half the size of the rabbit embryo. The modification of the trophoderm is still more precocious in the rat, for at 13 days in the rat the succession of plates and cell bodies is easily recognizable (fig. 6).

By referring to the figures given in former paragraphs (page 187), one can readily see that the dates of the development of the plates in the placenta correspond accurately with those of the involution of the Wolffian body, seen in the rabbit at about 21.0 mm. and in the guinea pig quite advanced at 15.0 mm. In the rat, since no glomeruli ever develop, the placental plates are found at about the period in which the mesonephric glomeruli become active in other mammals. The development of possible osmotic membranes in the placentae of various rodents at the precise time when such membranes are lacking in the corresponding embryos can hardly, it seems to me be a mere coincidence.

Another type of conjoined placenta is that found in most carnivora, of which the cat and the dog have usually been taken as types. A placental labyrinth is present, with what corresponds to the chorionic villi; the separate villi are not free, however, but are bound together by their trophoderm, through which the maternal blood has made a network of channels. Everywhere between the maternal blood stream and the fetal capillaries in the mesoderm of the villi the thick syncytial layer of the trophoderm intervenes. Nowhere does this layer become changed into membranous plates. At each edge of the zonate placenta there develops at about the middle of pregnancy the 'green column' of which mention has been made. Surprised at first by the very small excreting surface exhibited by the glomeruli of the Wolffian body and kidney of the cat, I sought in this 'green column' for a possible osmosis between fetal and maternal blood, but only to find that there is here no maternal circulation, merely an extravasation, and that no sign of a plate-like chorionic epithelium exists amid the peculiarly high columnar cells. In the

conjugate placenta of the cat, then, there is the same lack of provision for an osmotic interchange between mother and fetus as in the apposed placentae of the pig and the sheep. Thus in the placentae whether apposed or conjoined, of all the animals here studied which retain functional mesonephric glomeruli until the renal glomeruli are active, the thin plates of fetal epithelium are absent at all periods of gestation.

The placenta of man is of the conjoined type with free floating villi of vascular fetal mesoderm covered by an ectodermal epithelium. The nature of this epithelium is well known from the researches of many authors. At first it is of two layers, the inner cellular, the outer syncytial, with more deeply staining protoplasm and many nuclei. Originally very irregular, this syncytium assumes during the first and second months a more epithelial character, so that each villus shows clearly the two separate layers. Later degenerative processes set in; portions of the cellular layer disappear in places, leaving the syncytium as the only covering; the syncytium itself becomes changed in part into the 'cell knots' and 'canalized fibrin' of Minot. Degenerative vacuoles may appear in the syncytium later in pregnancy. The fetal capillaries approach nearer to the epithelium, running close to the bases of the still healthy portions of the syncytium, as they do in the active part of any gland. The surface of the chorion and its villi is so enormous in the human placenta that apparently much of it may become degenerated without destroying its functional ability to too great an extent, and the areas of functional epithelium may be widely separated by the inactive areas of canalized fibrin.

It is probably on account of the great surface area presented in the human placenta and the consequent utilization of only small portions of it for active functions that the membranous plates formed by the syncytial layer in conjunction with the fetal capillaries have not been heretofore mentioned or figured, to my knowledge. A little search is necessary to find the scattered plates, but they may be encountered in all parts of the labyrinth. Perhaps their relative infrequency, as compared with those found in the rodent placenta, is another expression of the small amount

of excretion in man, already noted in the paucity and small size of the mesonephric glomeruli. That they are actually present is shown in figures 5, 9, 10, and 11, drawings of the chorion and villi of the human placenta at different ages. Their extent in relation to the blood-vessels is also shown in figure 12, the drawing of a model of a placental villus from the placenta at term, for which I am indebted to Mr. Alan Gregg, a student of this School. The relation of fetal capillary, plate, and maternal blood stream is the same as in the rodent placenta, and that of capillary and plate the same as in any glomerulus. As in the glomerulus, here also the plates are sometimes obviously separate from the endothelium, sometimes so closely adherent as to appear fused with it into a single layer. The stretches of the thicker, granular protoplasm intervening between adjacent plates are longer in the placenta than in the glomerulus, because the cells, presumably of granular type, which supply nutrition, etc., to the fetus are present in the placental epithelium, but absent in the glomerulus.

The time of the appearance of these plates in the human placenta agrees sufficiently accurately with the time of the progressive degeneration of the mesonephric glomeruli in the embryo. They are found, as is shown in figure 5, in the chorion of the placenta of a fetus of 29.0 mm. Felix, as we have seen, places the end of mesonephric involution at 22.0 mm., which should be advanced slightly according to my own observations. The plates in the placenta may well be present even earlier; I have made no systematic search for them in closely graded stages. The plates increase progressively in number as pregnancy advances, and are quite striking at term. Their location seems to be gradually changed from the surface of the chorion in younger placentae to the smaller villi in older ones, which means, of course, that the early plates degenerate as new ones are constantly being formed.

We have seen that the placentae of all of that class of embryos in which we have found an early involution of the mesonephros exhibit membranous plates in the proper relation to the fetal vessels, and that the time of the disappearance of the one is

approximately the same as that of the appearance of the other. Moreover, the placentae of those animals studied which are able to utilize the Wolffian body until the kidney is ready for action show no such modification of the ectoderm. There are two objections, it seems to me, which might stand in the way of an immediate acceptance of these facts as proof that the placental plates surely represent the lost glomerular ones. The first has already been mentioned; since the 'breathing epithelium' of the lungs and of the gills is also characterized by membranous plates, may not the placental plates represent the apparatus for fetal oxygenation instead of for fetal excretion? As was pointed out previously, it is possible that the same apparatus may serve for both purposes. The strongest argument for considering the plates excretory instead of respiratory is the fact that all embryos from very early periods require oxygen, yet many classes of mammals never develop the placental plates, and in many others they appear relatively late, long after the need for oxygen must have been felt. In the rat alone, of those animals studied, would the development of the apparatus keep pace with the needs of the embryo. On the other hand, the fact that in the pig, sheep, and cat placental respiration must in all probability be an active secretory process does not eliminate the possibility of an osmotic respiration for other embryos at certain periods.

The second objection is perhaps a little more puzzling. If in certain cases the kidney becomes functional during fetal life, as we must suppose in animals provided with a large allantoic reservoir, and no placental osmotic apparatus, why should it not also become active in all mammals? In other words, why should excretion in certain animals take place through the placenta instead of through the kidney in the later periods of pregnancy, when the kidney is apparently capable of activity? Of the readiness of the older fetal kidneys of various classes of animals to perform the excretory function there is hardly a doubt. The kidney glomerulus of a new-born rabbit is quite similar to that of a new-born cat, though the one has not yet presumably been functional and the other has been active for many days.

In the case of man the question of the activity of the fetal kidney has been much considered by anatomists and physiologists, and many conflicting reports are published pro and con. The question is discussed by all those interested in the origin and constitution of the amniotic fluid. That the fetal kidney in man may be active just before birth is abundantly proved by the numerous observations of immediate micturition in the newborn, yet the small quantity passed in these cases and the absence of any great amount of the urinary constituents in the accompanying amniotic fluid show that the renal activity has not been of long duration. Ahlfeld and English demonstrate, by their cases of total absence of the fetal kidneys and of the blocking of the fetal urinary passages, already referred to, that the kidney is not a necessity before birth; on the other hand, cases of fetal hydronephrosis, of fetal calculi, and the occasional presence of large amounts of urinary matter in the amniotic fluid, all point to a prolonged renal activity. Preyer reviews the various statements and comes to the conclusion that the real cause of the inactivity of the fetal kidney is probably the low blood pressure of the fetus, and in proof of this contention quotes from Schatz a case of twins with separate amniotic cavities. One, which had an enormous amount of amniotic fluid, urinated a great quantity and almost hourly during the six hours of its life; the other, which had very little amniotic fluid, passed no urine in twelve hours. The kidneys and heart of the first weighed one and a half times as much as those of the second. The first had a much higher blood pressure, "liefert mehr Harn und dadurch mehr Fruchtwasser."⁷ Whether the fetal blood pressure of the pig, sheep, and cat, animals in which the fetal kidney is active, is relatively higher than that of rodents and man, in which it is not, I do not know; nor is it easy to understand the rise of fetal blood pressure within the placenta of rodents and man, to cause excretion there, which this explanation seems to call for.

Various experiments, quoted by Wertheimer, on rabbits and guinea pigs near term or in the latter part of pregnancy prove

⁷ Preyer, loc. cit., p. 333.

that the kidney of these animals also is capable of activity, but, as Wertheimer points out, this does not prove that excretion does normally take place, as in all of these experiments some disturbance of the fetal circulation is inevitable. The question, it seems to me, may be left open to further investigation, undertaken with a knowledge of the possibility of a placental excretion in certain animals.

CONCLUSIONS

The Wolffian body or mesonephros is a gland of urinary excretion.

Mammalian embryos may be divided into two classes; those which retain functional Wolffian bodies until the kidneys are sufficiently developed to excrete urine, as is the case in birds and reptiles, and those in which the Wolffian bodies degenerate before the kidneys reach functional ability. The first class includes the pig, sheep, and cat; the second, the rabbit, guinea pig, man, and rat.

Within each of these classes individual animals show great differences in the size and presumable excretory ability of the Wolffian bodies, without regard to the length of their duration.

The allantois is the receptacle of the urine formed within the body of the embryo; it is present as a reservoir only in those animals with an embryonic excretion, and its size varies with the size of the Wolffian bodies and with their duration. The urethral opening, though present, is not normally used for the passage of fetal urine.

In those animals without the possibility of a continuous urinary excretion within the embryo, i.e., with an early degeneration of the Wolffian body, the placenta is provided with an apparatus similar to that found in the glomeruli of the Wolffian body or the kidney, thin plates of epithelium overlying the fetal capillaries. These appear in the placenta at about the time when the Wolffian body commences to degenerate, or in the case of the rat, which never develops mesonephric glomeruli, at about the time of the normal development of the glomeruli in other embryos. These plates continue and increase in number till term. They

are apparently of greater extent in animals whose embryos are provided with large Wolffian bodies.

In the placentae of those animals with a continuous embryonic urinary excretion, similar plates are not found, whether the placentae be of the apposed or conjoined type.

From these facts it appears that embryonic and fetal urinary excretion takes place wholly through the placenta in the rat, at first through the Wolffian body and later through the placenta in the rabbit, guinea pig, and man, but never through the placenta in the pig, sheep, or cat. A knowledge of these differences should lead to more intelligent experiment on the permeability of the placenta.

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ABBREVIATIONS

| | |
|--|---|
| <i>b.l.</i> , basal layer of fetal ectoderm | <i>f.cap.</i> , fetal capillary |
| <i>end.</i> , endothelium | <i>m.b.s.</i> , maternal blood sinus |
| <i>ep.</i> , thicker, granular portion of epithelium | <i>pl.</i> , epithelial plate |
| | <i>syn.</i> , fetal syncytium, trophoderm |

PLATE I

EXPLANATION OF FIGURES

1 Portion of adult human renal glomerulus. Bowman's capsule and walls of convoluted tubules to the right of figure. Note the lobulation of the glomerulus, the epithelial plates covering the capillaries at the border of the capsular cavity, and the cell bodies, as at "ep." on this border between the plates. At "end." the plate and the underlying endothelium are each distinct. \times 640 diameters.

2 and 3 Portions of immature mesonephric glomeruli from a human embryo of about 10 mm. (Keibel, no. 1495.) To show development of epithelial plates and cell bodies. \times circa 640 diameters.

4 Portion of renal glomerulus of cat fetus of 8.5 cm. Orientation as in figure 1. Epithelial plates already present. \times 640 diameters.

6 Part of labyrinth of placenta of a rabbit of 27 days, showing the endothelium of the fetal capillaries and the succession of thin plates and thicker nucleated portions of the ectoderm, between capillary and maternal blood stream. Copied from Duval's Atlas, Placenta des Rongeurs, figure 62. \times 470 diameters.

7 Portion of placenta of a guinea pig of the second month, similar to figure 6. Copied from Duval's Atlas, figure 262. \times 300 diameters.

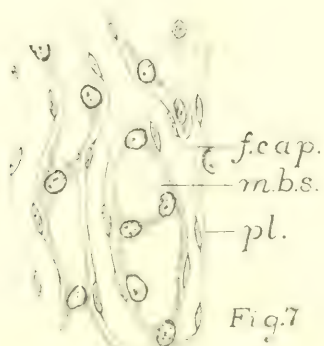
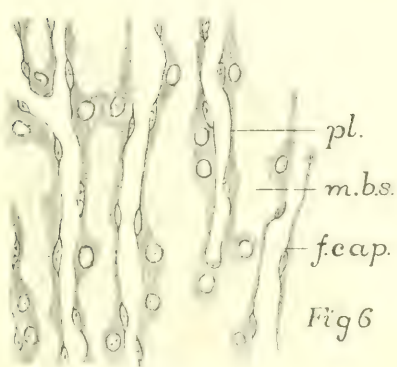
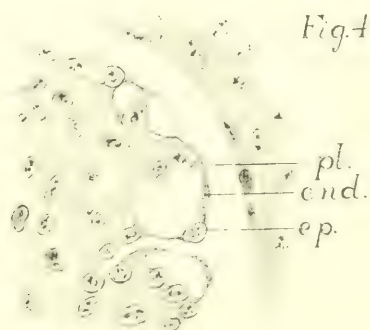
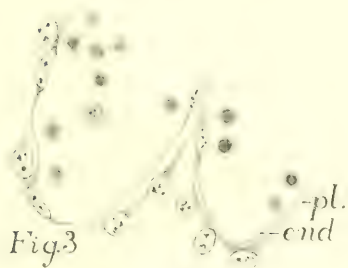
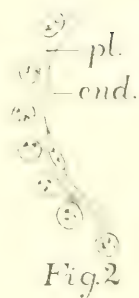
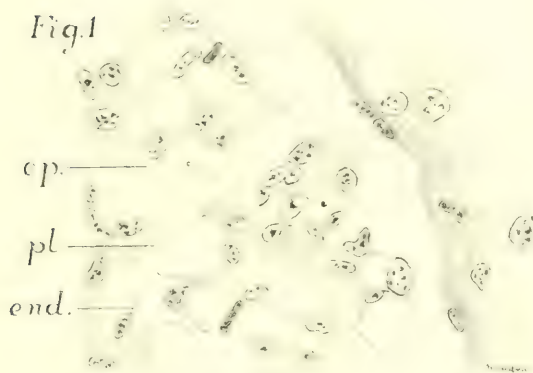


PLATE 2

EXPLANATION OF FIGURES

5 Portion of placental chorion of human embryo of 29.0 mm. (H. E. C. No. 389). Above, the chorionic mesoderm; the basal layer of the ectoderm and the syncytial layer are both interrupted by a fetal capillary, separated from the maternal blood stream only by an ectodermal plate, pl., which is closely adherent to the endothelium of the capillary. $\times 640$ diameters.

8 Portion of chorion and labyrinth of placenta of a rat of 13 days (H. E. C. no. 1930, sect. 143). The same production of epithelial plates separating the endothelium of the fetal capillaries from the maternal blood stream. The two streams are recognizable by their blood corpuscles. It will be noticed that the plates occur against both fetal arteries and veins. The basal layer of fetal ectoderm has partially disappeared. $\times 250$ diameters.

9 Villus of human placenta of 3 months. Note the complete syncytial layer of the fetal ectoderm, and the basal layer interrupted by a fetal capillary, over which the syncytium has developed a plate. $\times 480$ diameters.

10 and 11 Villi of human placenta at term. The basal layer of ectoderm is no longer present. The syncytial layer shows a succession of thick granular nucleated portions and thin epithelial plates in direct contact with the fetal capillaries. The maternal blood stream surrounds the villi. $\times 480$ diameters.

12 Model of the blood-vessels and the ectodermal syncytium of a villus of the human placenta at term. It will be noticed that two small villi have fused, making a ring formation, around which capillaries pass. One artery and two veins pass into the villus. In addition to the areas seen in profile where the ectodermal covering is of plate-like thinness, the blood-vessels are also covered by plates between *x* and *x*, and at *y*, and *z*. This, with figure 11, shows the relative extent of the plates and the thicker syncytium. $\times 250$ diameters.

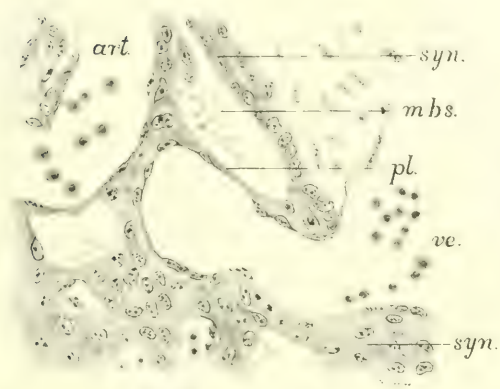


Fig. 8

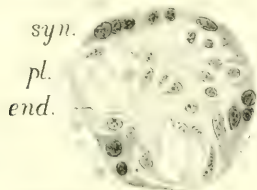


Fig. 10



Fig. 12

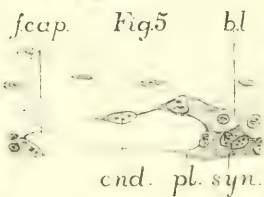


Fig. 5

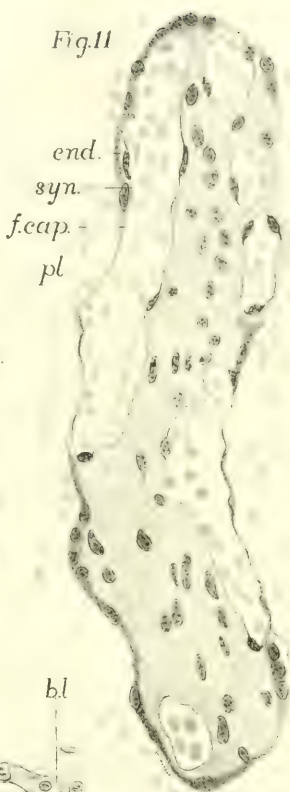


Fig. 11



Fig. 9

THE DEVELOPMENT OF THE LIVER AND PANCREAS IN AMBLYSTOMA PUNCTATUM

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FORTY-SIX FIGURES (FOUR PLATES)

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I. INTRODUCTION

Comparatively little work has been done on the morphology of the biliary and pancreatic duct-systems in vertebrates. The arrangement of these structures has been worked out in the adult forms of a few species but no attempt has been made to correlate these scattered observations or to determine what may be considered the typical arrangement in vertebrates and the major variations which may occur in the various groups of the phylum. The development of these systems is also

almost unknown. Although the formation of the anlagen of the liver and pancreas has been investigated in almost every group of vertebrates, the later history of duct systems of these structures has been quite neglected. The two exceptions to this statement are furnished by the work of Corner ('13) who investigated the development of the pancreatic ducts of the pig by means of injection methods and Scammon's study of the biliary system of selachians.¹

The following study is an attempt to follow in detail the development of these duct-systems in the tailed amphibia, and to point out the embryologic significance of the principal variations which are encountered in the adult and the mechanical influences which are, in part at least, responsible for them. Although we are not as yet in possession of sufficient data to formulate a statement of the typical vertebrate plan of biliary and pancreatic duct-systems, it is hoped that this description of these structures in a representative amphibian may add to the material upon which such a schema must eventually be based.

The material used for this work consisted of embryos of *Amblystoma punctatum* from 4 mm. to 20 cm. in length. These were sectioned serially in transverse and sagittal planes. Graphic and wax reconstructions were made of the hepatic ducts, gall-bladder, liver and pancreas of different embryos and adults.

It is a pleasure to express my thanks to Dr. Richard E. Scammon for his constant interest and helpful criticisms throughout this study.

A correlation of the embryos employed in this study with those described in the Normal Plates of *Necturus maculosus* by Eycleshymer and Wilson may be desirable. This correlation is based on a comparison of the digestive system including liver and pancreas, as well as partially on the external form. Probably the greatest difference in the development of the digestive tract between these two forms is in the time of union of the dorsal and ventral pancreatic anlagen which had taken

¹ The terms used by Scammon ('13) in describing the ducts of Elasmobranchs have been used in this paper.

place in most of the 13 mm. *Amblystoma* embryos which I have observed, and is described in stage 42 (29 mm.) in the Normal Plate series of *Necturus*. Also the limbs, particularly the caudal ones, appear comparatively later in *Amblystoma*. Such a table, of course, can be only an approximate comparison.

TABLE 1

Correlation of Amblystoma embryos with the Normal-plate series of Necturus

| FIGURES | | EMBRYOS | | NORMAL-PLATE SERIES | |
|---------------------|--|---------------|--|---------------------|---------------|
| Figure | | Length in mm. | | Stage No. | Length in mm. |
| 1..... | | 4.5 | | 21 | 8 |
| 2..... | | 5 | | 22-23 | 9 |
| 3..... | | 7 | | 25 | 12 |
| 4..... | | 9 | | 28 | 15 |
| 5..... | | 9 | | 29 | 16 |
| 27-30..... | | 11 | | 30 | 17 |
| 6..... | | 11.5 | | 31 | 18 |
| 21..... | | 12.5 | | 34 | 21 |
| 31, 45..... | | 13 | | | |
| 18..... | | 13 | | 38 | 25 |
| 19..... | | 13.5 | | 39 | 26 |
| 8, 39, 40..... | | 14 | | 42 | 29 |
| 7A, 9, 41..... | | 13.5 | | 43 | 30 |
| 10, 33, 42, 46..... | | 15 | | 45 | 32 |
| 11, 20, 43..... | | 20 | | 49 | 39 |

II. THE DEVELOPMENT OF THE LIVER, HEPATIC DUCTS AND GALL-BLADDER

1. Literature

The literature of the development of the great glands of the digestive tract of Amphibia can be conveniently divided into two parts covering two fairly distinct periods: first, the work of the early investigators who determined the position of these glands in the embryo and their relation to the lower germ layer; second, the series of contributions beginning with Goette's large monograph upon the development of Bombinator ('75) and dealing mainly with the detailed developmental anatomy of these organs.

The following table gives a list of the authors, the dates of their publications and the material upon which their work on the development of the liver and pancreas was based.

Steinheim ('20) studied older embryos and observed the attachment to the gut. Rusconi ('26) investigated younger embryos and, as did Reichert ('40) and Vogt ('42), described the ventral growth of the intestine to form the liver. Remak ('55) and v. Bambecke ('68) differed from the above only in the number of lobes formed and noted the close relation of the gall-bladder to the right lobe.

According to Goette ('75), the liver in *Bombinator* originates as a ventral outpouching of the foregut posterior to the heart. This diverticulum becomes separated from the gut by a gradual cranio-caudal constriction, and the narrow connection which remains forms the common hepatic duct. The outpouching then grows by the production of folds or buds from its sides which form the primary hepatic columns. The lumina remain in these columns although they may be very small. Goette regarded the early anastomoses and formation of the net-like hepatic cylinders as aided by the ingrowth of a capillary network. The gall-bladder develops as an outpouching of the posterior part of the primitive hepatic duct caudal to which the ductus choledochus is formed.

Balfour ('81) made the statement that there is a single ventral diverticulum from the gut which later develops into two secondary branches and so forms the liver.

Shore ('91) in his study on the frog found that the liver takes origin as a ventral lengthening of the gut lumen into the mass of yolk-cells which lies posterior to the heart. The yolk-cells lining this lumen are transformed into hepatic cells and this mass becomes partially separated from the gut. This constriction is aided by the caudal growth of the sinus venosus. Later there is formed at the expense of the yolk-cells and by cell-division a large cell-mass into which the blood-vessels tunnel forming a tubular gland whose columns divide and anastomose producing a network interlacing with that of 'blood-lacunae.'

Marshall ('93) gave a brief account of the development of the liver in the frog in his vertebrate embryology. He described a caudo-ventral projection from the anterior part of the mesen-

TABLE 2
Table of authors and the forms studied

| AUTHOR | DATE | MATERIAL |
|----------------------------------|------|--|
| Steinheim..... | 1820 | Rana |
| Rusconi..... | 1826 | Rana |
| Reichert..... | 1840 | Rana temporaria Rana esculenta |
| Rusconi..... | 1854 | Salamandra |
| Vogt..... | 1842 | Alytes obstetricans |
| Remak..... | 1855 | Rana temporaria Rana esculenta |
| Rathke..... | 1861 | "Vertebrates" |
| Bambecke..... | 1868 | Pelobates fuscus |
| Goette..... | 1875 | Bombinator igneus |
| Wiedersheim..... | 1875 | Salamandra perspicillata |
| Balfour..... | 1881 | "Amphibia" |
| Shore..... | 1891 | Rana |
| Goepfert..... | 1891 | Salamandra maculata, etc. Bufo vulgaris, etc. |
| Marshall..... | 1893 | Rana |
| Minot..... | 1893 | "Amphibia" |
| Weyse..... | 1895 | Rana temporaria Rana esculenta |
| Stöhr..... | 1895 | Rana temporaria |
| Hertwig..... | 1896 | "Amphibia" |
| Brachet..... | 1896 | Review |
| Hammar..... | 1897 | Rana |
| Woit..... | 1897 | Rana temporaria Triton taeniatus, etc. |
| Kollmann..... | 1898 | "Amphibia" |
| Gianelli..... | 1899 | Triton cristatus |
| Choronshitzky..... | 1900 | Rana temporaria Salamandra maculosa, etc. |
| Reuter..... | 1900 | Alytes obstetricans |
| Gianelli..... | 1902 | Triton |
| Piper..... | 1902 | Review |
| Weber..... | 1903 | Review. |
| Braun..... | 1906 | Alytes obstetricans |
| Eycleshymer a n d Wilson..... | 1910 | Necturus maculosus |
| Baumgartner..... | 1914 | Amblystoma punctatum |

teron. The anterior wall of this depression is thrown into folds, blood-vessels penetrate between these structures and outgrowths from the hypoblast form the hepatic cylinders.

Weyssse ('95) found in the frog that the liver-anlage is a dorso-ventral cleft extending into the yolk-mass from the gut lumen. A caudal extension of this cleft forms the posterior hepatic duct, while the cranial hepatic duct is formed by a folding of the anterior wall of the hepatic anlage. The yolk-cells are transformed into the true hepatic cells and can be early recognized by the deposit of pigment within them.

Hertwig ('96) and Kollman ('98) gave only short descriptions, stating that in *Amphibia* the hepatic anlage is a single out-pouching from the ventral wall of the duodenum.

Hammar ('97) who worked on the development of the frog's liver, has named the entodermal cell-mass posterior to the heart the 'Leberprominenz.' Into this extends an early lengthening cavity which is continuous with the lumen of the gut. This he termed the 'Leberbucht.' By a cranio-caudal constriction this hepatic anlage is separated from the gut. The cell-mass about the fundus of this anteriorly directed sac develops into trabeculae of the adult organ and the posterior part forms the ductus choledochus. The gall-bladder is developed very early as a diverticulum of the ventral wall of the common bile duct, and by further growth comes to be a pedunculated organ, consisting of a cystic duct and gall-bladder proper. He regarded the origin of the trabeculae as perhaps due partially to the developing capillary network tunnelling into the hepatic cell mass as suggested by Shore.

Choronshitzky ('00) showed the anlage of the liver in the salamander in a figure of a sagittal section of a 9 mm. embryo, in which there is a ventral fold in the wall of the foregut. This fold is lined with yolk-laden cylindrical cells which posteriorly pass gradually over into the polygonal yolk-cells which form a mass projecting into the lumen of the gut. In the anterior ventral wall of the gut is a second slight pouch which later forms the gall-bladder. The two omphalo-mesenteric veins crowd in on either side of the liver outpouching, thereby aid-

ing the constriction of the lateral walls of the gut. These veins unite anteriorly and form the ductus venosus. The liver-anlage therefore first grows ventrally and then anteriorly below the horseshoe-shaped union of the omphalo-mesenteric veins and the ductus venosus. A similar sagittal section of a later stage shows the liver at the cranial end of a short ductus hepaticus which is continuous caudally with the ductus choledochus. From the ventral wall of the ductus choledochus there is now a very marked outpouching, the gall-bladder, which is united with the common duct by a short cystic duct. The primitive liver-anlage has thus grown cranialward and become separated from the gut. Choronskitkzy believes this process to be due to the growth and differentiation of the gut. The walls of the primitive liver-anlage have folded and these folds later develop into solid liver-columns. The liver grows around the developing ductus venosus even to its dorsal surface and in so doing produces many folds and columns which grow through the ductus venosus and divide it into sinus-like branches.

Reuter ('00) in his studies on the development of the intestine of the *Alytes obstetricans* made mention of the early origin of the liver. This develops from the 'Anfangsdarm' division of the midgut. In later embryos the liver develops very rapidly and is divided into three lobes.

Gianelli ('01 and '02) described the hepatic anlage in *Triton* as developing in two parts, the anterior giving rise to the hepatic tissue proper and the caudal forming the hepatic duct. The gall-bladder arises from a mass of cells belonging to the primitive hepatic outpouching. By the development of the intestinal folds the hepatic duct becomes attached to the dorsal side of the gut.

Weber ('03) stated that the observations made on the development of the liver in the frog and in *Triton* differ but little. In the latter the intimate relation of the anterior end of the hepatic outpouching and the blood-vessels account for the development of this part into the hepatic tissue proper.

Bates ('04) in a paper on the histology of the digestive tract of *Amblystoma* has described the hepatic and pancreatic ducts.

He has described a bile-duct which lies free in the body-cavity for a short distance and then enters the pancreas which lies between the liver and the intestine. Here it is joined by two hepatic ducts and just as this enters the intestine it is joined by two other hepatic ducts.

To summarize briefly, the early investigators described the liver and pancreas as developing at the same time from the ventral wall of the gut, and also considered that they were parts or lobes of the same organ. Remak ('55) first noted that the liver is separate and distinct from the pancreas. Goette first gave a detailed account of the development of the liver in amphibia. Most of the investigators from that time have agreed that the liver begins as a single ventral outpouching of the gut-wall caudal to the heart. The question as to the origin of the gall-bladder, whether from the caudal end of the ductus choledochus or from the wall of the intestine in this region may be, as Piper ('02) stated, one of interpretation rather than one of observation. Whether the hepatic cylinders divide and the blood-capillaries then grow between them, or whether the capillaries grow into the solid hepatic anlage so forming hepatic cylinders seems not to have been definitely determined. Shore's ('91) observations support the latter theory. According to the observations of Weyssse and others the yolk-cells are transformed directly into hepatic cells. Very little has been written about the development of the hepatic ducts. The common bile-duct is described as the constricted attachment of the hepatic anlage, or the posterior end of the hepatic outpouching.

2. Early development of the liver

The liver in *Amblystoma* first appears in embryos about 4.5 mm. in length, which corresponds roughly to no. 21 of Keibel's Normal-plate series. The digestive tract at this stage is quite simple. The pharyngeal cavity is large and extends anteriorly to the oral cavity. Caudally it opens widely into the mesenteron which is composed of a large mass of yolk-cells and extends backward to the proctodaeum. The yolk-mass extends dorsally to the notochord and bulges ventrally.

Posterior to the anlage of the heart a sagittal section shows a ventrally and somewhat caudally directed projection of the gut-lumen, (fig. 1) which extends backward near the dorsal side of the yolk-mass. The anterior wall of the ventrally directed extension of the gut-lumen is lined by yolk-laden columnar cells and its posterior wall is formed by the cells of the large yolk-mass. This cavity is quite wide transversely and is connected to the gut-lumen above by a wide cleft.

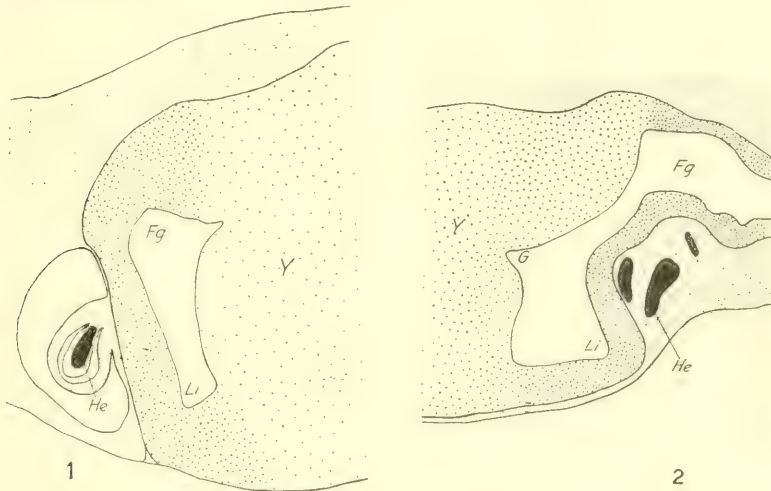


Fig. 1 Sagittal section of an *Amblystoma* embryo 4.5 mm. long taken at about the median plane. $\times 30$. *F.g.*, foregut; *He*, heart; *Li*, liver; *Y*, yolk mass.

Fig. 2 Sagittal section of an *Amblystoma* embryo 5 mm. long, taken to the right of the median line. $\times 30$. *F.g.*, foregut; *G*, caudal extension of gut; *He*, heart; *Li*, liver; *Y*, yolk mass.

Weyssé ('95) has described this cavity in frog as a cleft in the ventral mass of yolk-cells, and Hammar ('97) has termed it the 'Leberbucht.' From the study of a slightly more advanced stage Weyssé concluded that the caudal and ventral end of this cleft finally formed a caudal hepatic duct. He correlated this with the caudal hepatic duct described in the chick. That the caudal projection does not form a caudal hepatic duct in amphibia seems clear from a study of the later development. The reason for this error was probably, as Hammar has pointed out, that

Weyssse did not follow the development beyond a very early stage.

In an embryo approximately 5 mm. (fig. 2) long the anterior wall of this early ventro-caudal projecting cavity has become more prominent. The extension of the gut-lumen into this out-pouching is a large cone-shaped cavity somewhat flattened in transection. The columnar epithelial cells lining it are now found farther caudalward than in the preceding stage.

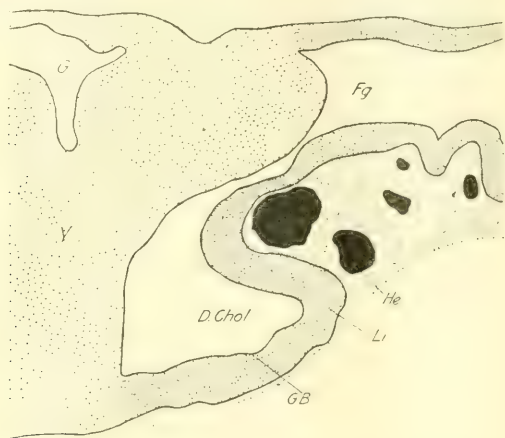


Fig. 3 Sagittal section of an embryo almost 7 mm. long. $\times 30$. *D.chol.*, ductus choledochus; *F.g.*, foregut; *G.*, caudal extension of gut; *G.B.*, gall-bladder; *He*, heart; *Li*, liver; *Y*, yolk mass.

In a sagittal section of an embryo 7 mm. long there is shown a more advanced stage of the condition just described. From a comparison of this stage (fig. 3) with the previous one and the one following, it will be seen that the hepatic anlage has become more prominent by a cranio-caudal constriction from the gut. Folds have begun to form on the outer surface of the liver. The cavity of the hepatic diverticulum is widely connected with that of the gut. In the ventral wall there is a slight median depression (*GB*) which is the earliest indication of the gall-bladder. This depression is at the caudal end of the liver-anlage in the region where the primitive ductus choledochus is forming.

The liver of another embryo 7 mm. long appears as an anterior and ventral outpouching of the gut. Figure 36 is of a plastic reconstruction of this region of the archenteron. That the constriction from the gut has proceeded caudally will be apparent by comparison with earlier and later stages. The cavity projecting into the liver-anlage from the lumen of the gut is now much longer, and there are indications of further projections from it on the right side as the lumina of ducts.

Choronshitzky noted this transverse extension of the lumen in the hepatic anlage of the salamander but did not follow its further history. At the posterior end in the median ventral wall is a marked outpouching which is the gall-bladder (*GB*, fig. 36). The opening of this outpouching into the gut is still very wide laterally and shows no differentiation into cystic duct and gall-bladder. The evagination is wide transversely though not extending as far laterally as the liver. In ventral view the gall-bladder appears as a wide transverse outpouching. There is a slight furrow separating it anteriorly and laterally from the liver proper, and a more pronounced one separating it from the caudally placed yolk-mass.

In an embryo approximately 9 mm. in length (fig. 37) the liver is distinctly further advanced than in the preceding one. The caudal constriction from the gut has progressed rapidly (fig. 4). The original anterior convex surface of the liver has become markedly irregular showing numerous depressions or furrows between projecting masses of cells. Greil ('05) figures a model of the liver in a Bombinator embryo 7.5 mm. long with many secondary buds. A network of veins already occupies the spaces between the hepatic buds but Greil only states that it is present. The anteriorly directed cavity has become constricted dorso-ventrally and the division into ducts is more distinct. On the left side (fig. 37) there is a ventral (*vl*) and a dorsal (*dm*) projection of the lumen. On the right side the ventro-lateral extension is prominent. The median ventral evagination (*GB*) has become more pronounced. There is now the beginning of a lateral constriction of this evagination representing the formation of a cystic duct. The anterior lip of the

evagination has developed into quite a ridge separating the gall-bladder from the developing hepatic ducts. On the ventral surface the anterior furrow separating liver and gall-bladder from yolk-mass is, as before, the more marked.

According to Shore ('91) in the frog the furrows found in the liver-mass are caused by the 'tunnelling in' of blood vessels. That it is not due only to this is apparent in *Amblystoma* where sections of this and other embryos show furrows in which there are no blood-vessels (fig. 4). It is important to note that Shore saw no vascular endothelium in these spaces which he regarded as blood-vessels.

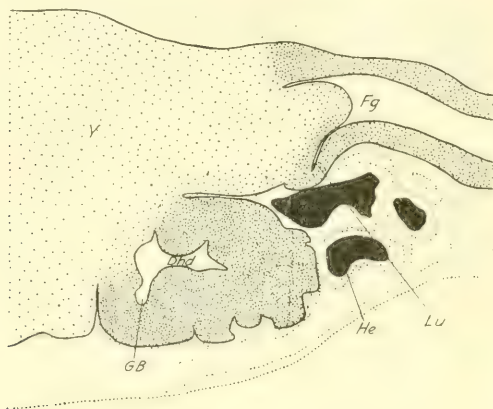


Fig. 4 Sagittal section of an embryo almost 9 mm. long. $\times 30$. *Dhd.*, ductus choledochus; *F.g.*, foregut; *G.B.*, gall-bladder; *He*, heart; *Li.*, liver; *Lu.*, lung; *Y*, yolk mass.

In another embryo of 9 mm. in length the liver in cross section (fig. 5) appears as a large oval mass with an irregular surface showing deep furrows separating the developing ducts. There is also a very marked dorso-ventral furrow separating the liver-mass into two unequal lateral portions of which the left is the smaller. The right portion is marked by two lesser furrows, one ventral, the other lateral.

In 10 mm. embryos a beginning of the network of anastomosing trabeculae can be seen. The development of the sinusoidal

capillary circulation in this network has progressed. In the 11 and 12 mm. embryos there is a confusing network of trabeculae and it is difficult to differentiate the main ducts from the hepatic columns. Shore believed that in the frog the tubules were first solid and that later a lumen developed. Goette expressed the opinion that a lumen was present from the earliest formation, though he admitted this was hard to demonstrate. The reason of the difficulty of proving this either way is apparent. However, from a study of sections of *Amblystoma* it would seem that a lumen is present from the earliest stages.

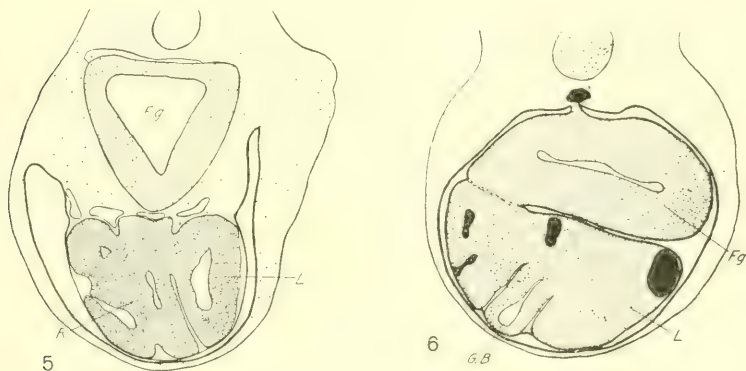
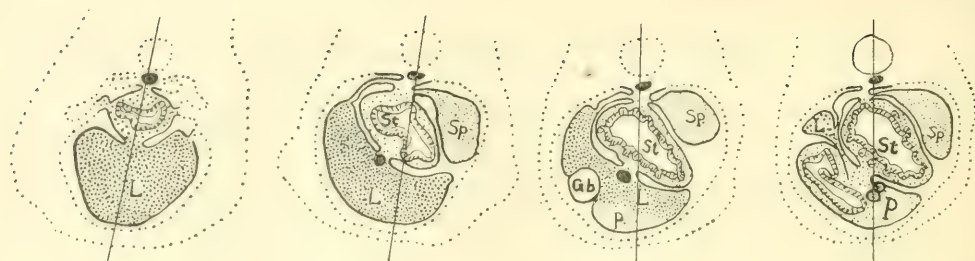


Fig. 5 Transverse section of embryo 9 mm. long. $\times 30$. *F.g.*, foregut; *L*, left portion liver; *R*, right portion liver.

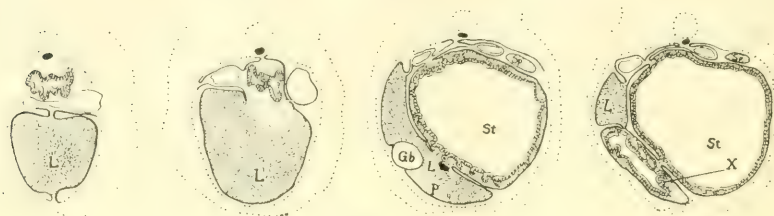
Fig. 6 Transverse section of an embryo 11.5 mm. long. $\times 30$. *F.g.*, foregut; *GB.*, gall-bladder; *L.*, liver.

3. Position of the organ during development

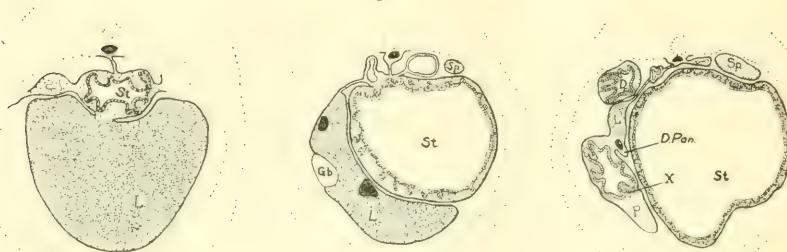
At a stage represented by 11.5 mm. embryos there is a shifting to the right particularly of the caudal end of the liver (fig. 6). Such a shifting of the posterior part of the liver was noted at a later stage in *Neoturus* by Eyeleshymer and Wilson ('10) and others. The reason for this lateralward shifting is probably the pressure of the rapidly growing stomach and duodenum which are beginning to take a ventral and sinistral position. It is possible also that the spleen which is now a prominent organ in the left dorsal region of the body cavity has some influence on this



7A



7B



7C

Fig. 7 A series of transverse sections in the region of the liver. A, embryo of 13.5 mm. $\times 20$; B, embryo of 20 mm. $\times 15$; C, embryo of 35 mm. $\times 10$; G.b., gall-bladder; L, liver; P., pancreas; Sp., spleen; St., stomach; x, ostia of ductus choledochus into gut.

| | At level of anterior end of liver | About midway between first and third drawing | Anterior end of gall bladder | Level of attachment of cystic duct to gall bladder | Level of ostium of ductus choledochus |
|-------------|-----------------------------------|--|------------------------------|--|---------------------------------------|
| A 13.5..... | " | " | " | Fig. 9 | " |
| 14 mm. | — | — | — | Fig. 8 | — |
| 15 mm. | — | — | — | Fig. 10 | — |
| B 20..... | " | " | " | Fig. 11 | " |
| C 35..... | " | — | " | Fig. 12 | " |

movement. Then, too, the ventral pancreas forms quite a mass in the median ventral region. Figures 7 A, B and C show the lateral and upward shifting of the posterior portion of the liver. The first drawing in each of the series shows a section taken near the anterior end of the liver which here is median and ventral in position and occupies somewhat more than one-half of the area of a circle. The second drawings in figure 7 A and B show a beginning of a depression on the left side caused largely by the change in shape and position of the stomach and duodenum as mentioned above. Figures 8 to 12 are cross sections of embryos 13.5 to 35 mm. in length showing the position of the liver at the level of the junction of gall-bladder and cystic ducts. Here the lateral and dorsal growth of the liver is marked. A somewhat further shifting is shown in the third drawing of figure 7 A and B and the second of 7 C. These sections were taken near the anterior extremity of the gall-bladder. In all of these the liver is crescentic in transsection and extends upward almost to the level of the dorsal wall of the stomach. The last drawing in figure 7 shows the relation of parts at the level of the opening of the ductus choledochus in the gut. In all cases a small portion of the liver is found dorsal to the duodenum in this region of the embryo. In an embryo 45 mm. long the anterior end of the liver is median and ventral as described above. There is a marked lateral and dorsal growth of the caudal end but in this embryo there is also quite a marked ventral growth which would indicate that from now on the shifting to the right will not be so noticeable, and that there is a growth to the left also.

4. *Development of the biliary apparatus*

a. *Description of the hepatic ducts in the adult.* A description of the fully formed biliary apparatus may be of interest before describing the development of the hepatic ducts.

The liver in the adult *Amblystoma* is a large organ extending fully one-half the length of the abdominal cavity (fig. 13). It has a ventral convex surface conforming to the wall of the abdomen

and is divided by an indefinite median line into a right and a left part of which the left is the longer and covers the left ventral surface and a part of the lateral wall of the stomach. The right portion or lobe though somewhat shorter, covers the ven-

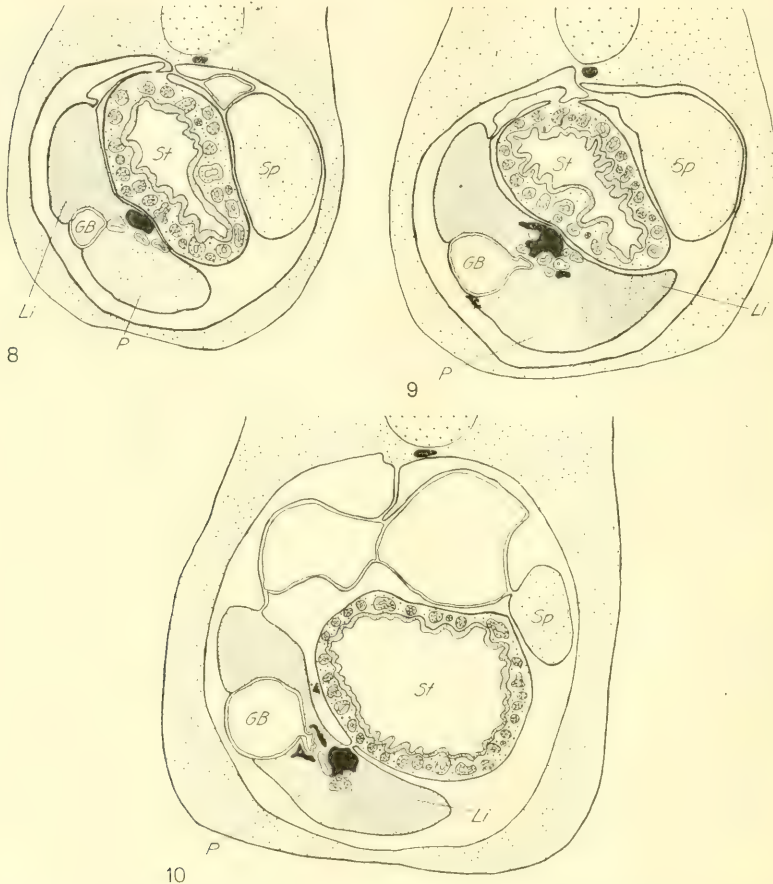
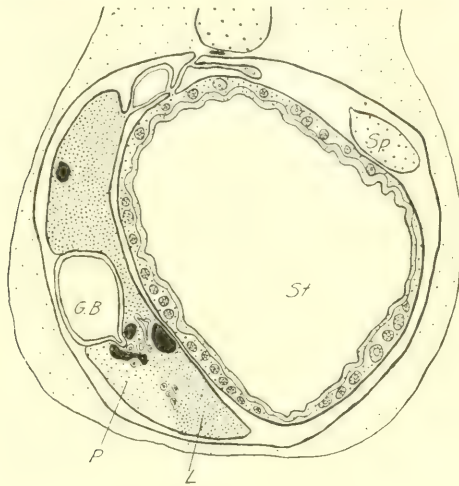


Fig. 8 Transverse sections of an *Amblystoma* embryo 14 mm. long, taken at level of attachment of cystic duct to the gall-bladder. $\times 35$. *D*, duodenum; *G.B.*, gall-bladder; *Li.*, liver; *P.*, pancreas; *Sp.*, spleen; *St.*, stomach.

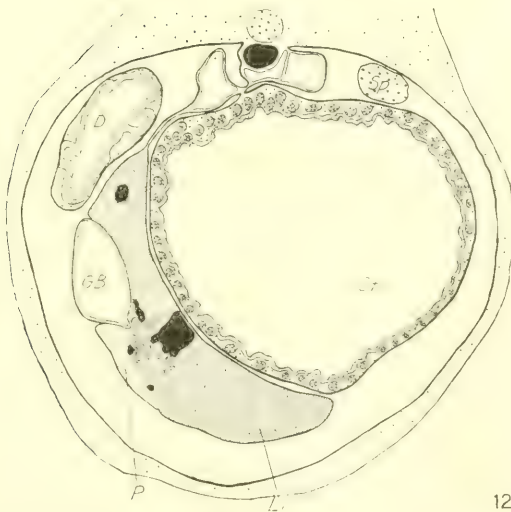
Fig. 9 Transverse section of an *Amblystoma* embryo 13.5 mm. long, taken at the same level as figure 8. $\times 35$. For abbreviations, see figure 8.

Fig. 10 Transverse section of an embryo 15 mm. long, taken at the same level as figure 8. $\times 35$. For abbreviations see figure 8.

tral surface of the stomach to the right of the midline and laterally extends well toward the dorsal wall of the stomach. There



II



12

Fig. 11 Transverse section of an embryo 20 mm. long, taken at the same level as figure 8. $\times 30$. For abbreviations see figure 8.

Fig. 12 Transverse section of an embryo 35 mm. long, taken as in figure 8. $\times 15$. For abbreviations, see figure 8.

are usually one or two lesser indefinite furrows dividing the right lobe into two or three parts. The gall-bladder is embedded in the caudal end of the right lobe some distance from its ventral surface. Only a small part of its rounded fundus appears beyond the hepatic tissue. From the notch in the liver caused by the gall-bladder the one or two lesser furrows of the right lobe extend forward. The gall-bladder is a pear-shaped sac with its larger end extending laterally and somewhat pos-

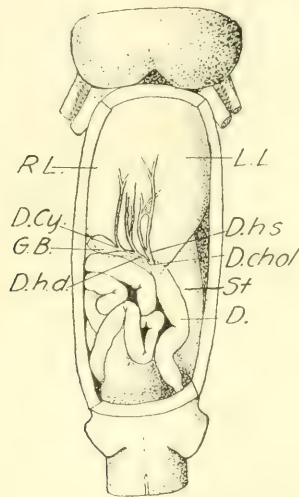


Fig. 13 A dissection of an *Amblystoma* 12 cm. long. $\times 1$. The ventral abdominal wall has been cut away and the gall bladder and main hepatic ducts dissected out. *D.*, duodenum; *D.chol.*, ductus choledochus; *D.cy.*, cystic duct; *D.h.d.*, right hepatic duct; *D.h.s.*, left hepatic duct; *L.L.*, left lobe liver; *R.L.*, right lobe liver; *St.*, stomach.

teriorly. The smaller, medial and ventral end projects forward and connects with the short cystic duct. Only the large blind end of the gall-bladder receives a peritoneal covering, the remainder is embedded in hepatic tissue.

There are two main hepatic ducts. These unite to form a common bile-duct of variable length which may be joined by the pancreatic duct just before opening into the gut (fig. 14). Quite often, however, the pancreatic duct opened into the gut immediately beside the ostium of the common bile-duct. The ductus

choledochus is embedded for some distance in the long narrow pancreas lying on the anterior surface of the duodenum and finally empties into the anterior side of the gut near the ventral surface.

The right hepatic duct is divided into lateral and medial rami. The lateral ramus divides into medial and lateral branches. Generally the cystic duct opens into the latter (fig. 14 and 16). However, sometimes the cystic duct is one or

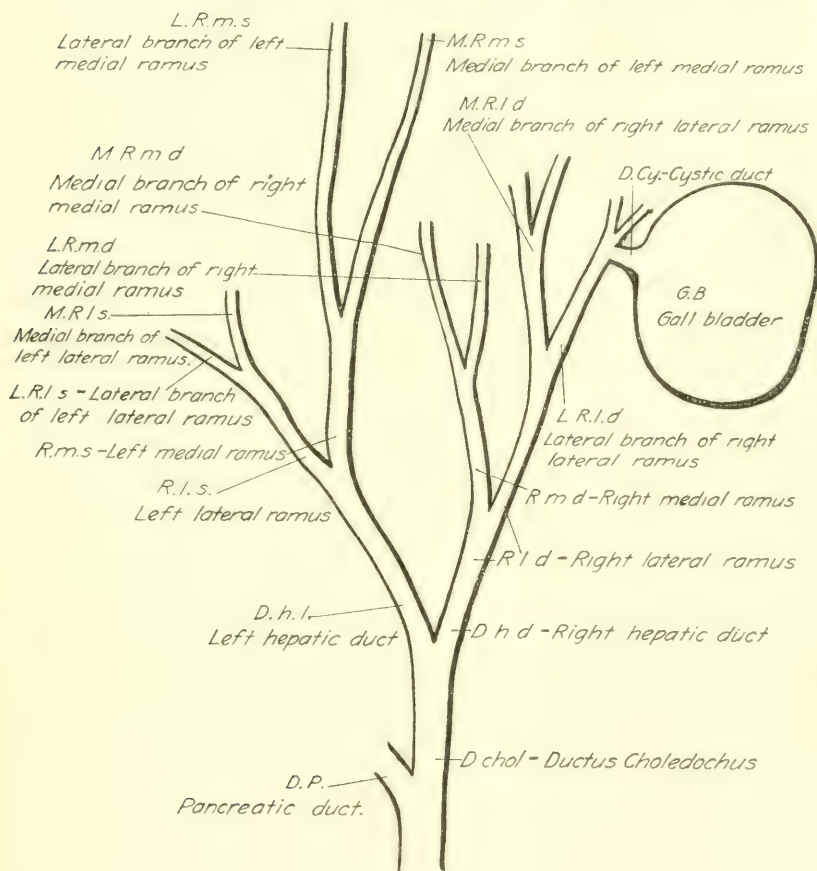


Fig. 14 Diagrammatic drawing of the gall-bladder and hepatic ducts of an *Amblystoma*.

even two divisions further removed from the common duct, as shown in figure 17 and 44. In a graphic reconstruction of the biliary apparatus of a 7 cm. embryo (fig. 15) the cystic duct joins the right lateral ramus as is shown also in figure 43. The hepatic radicle to which the cystic duct is attached shortly divides into trabeculae beyond this point. The right medial hepatic ramus divides and subdivides into branches as shown in figure 14. Its branches sometimes anastomose with the branches of the right lateral or left medial ramus (fig. 17).

The left hepatic duct is generally shorter and of slightly smaller diameter than the right one, as well as more ventral in position. It is divided as the latter into lateral and medial rami. The left medial ramus sometimes joins the right medial ramus as shown in figure 16, and this duct then subdivides as a single one. Frequently, however, the left medial ramus runs anteriorly subdividing into smaller branches of which some may anastomose with those of the right medial (fig. 17). The left lateral ramus is shortly divided into two of which the lateral either turns caudally (fig. 44) or sends out branches that go to the posterior portion of the longer left lobe.

b. Development of the ductus choledochus. The ductus choledochus in 9 mm. embryos is still very wide and short. The original caudalward projection from the gut cavity has disappeared and there is only the anteriorly directed common duct. In a model of an embryo 9 mm. long the ductus choledochus is wide transversely but constricted dorso-ventrally (fig. 37 and 38). It is attached at the anterior side of the now ventrally directed gut. At 11 mm. the duodenum has turned ventrally and folded to the right. A very much constricted and short common duct is attached to its superior anterior surface. In a 13 mm. embryo the common duct is attached to the anterior surface of the cranial fold of the duodenum. As before, the duct is small and short, soon dividing into right and left hepatic ducts. The epithelial lining of the duct still contains yolk-granules and except for a quite irregular but prominent lumen is very much like the hepatic ducts. Indeed the difference in the

lining cells of this duct and those of the hepatic trabeculae is not great.

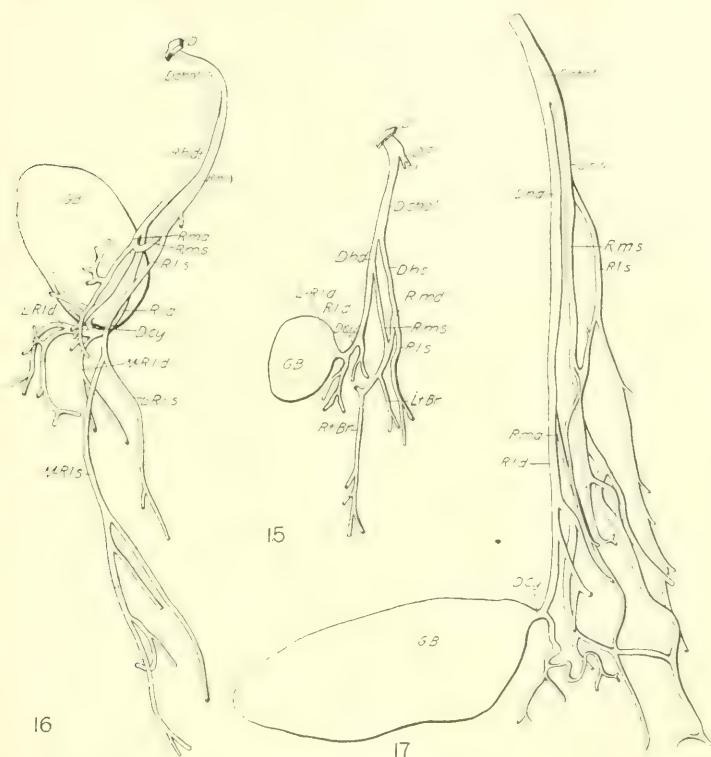


Fig. 15 Graphic reconstruction (lateral view) of an *Amblystoma* 7 cm. long. $\times 15$. *D.chol.*, ductus choledochus; *D.cy.*, cystic duct; *D.h.d.*, right hepatic duct; *D.h.s.*, left hepatic duct; *D.P.*, pancreatic duct; *G.B.*, gall-bladder; *L.Br.*, left branch of common ramus; *L.R.l.d.*, lateral branch right lateral ramus; *L.R.l.s.*, lateral branch left lateral ramus; *L.R.m.d.*, lateral branch right medial ramus; *L.R.m.s.*, lateral branch left medial ramus; *M.R.l.d.*, medial branch right lateral ramus; *M.R.l.s.*, medial branch left lateral ramus; *M.R.m.d.*, medial branch right medial ramus; *M.R.m.s.*, medial branch left medial ramus; *R.Br.*, right branch of common ramus; *R.l.d.*, right lateral ramus; *R.l.s.*, left lateral ramus; *R.m.d.*, right medial ramus; *R.m.s.*, left medial ramus.

Fig. 16 Graphic reconstruction (ventral view) of an *Amblystoma* 10 cm. long. $\times 15$. For abbreviations, see figure 15.

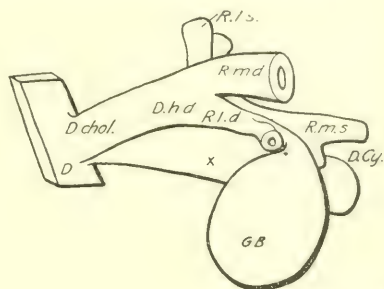
Fig. 17 Graphic reconstruction (lateral view) of an *Amblystoma* 15 cm. long. $\times 15$. For abbreviations, see figure 15.

In another embryo of approximately 13 mm. length, which is somewhat more advanced, the ductus choledochus is longer and of larger caliber (figs. 18 and 19). It is, however, still attached to the cranial surface of the anterior fold of the duodenum. The epithelium here is now definitely columnar in type, though yolk-granules are still present. In this case the pancreatic duct is attached near the gut to the common duct.² In an embryo 13.5 mm. long the ductus choledochus (fig. 7-A) is attached in a fold to the left side of the gut. The duct here is large but shortly divides into the right and left hepatic ducts. The attachment of the duct to the left wall of the gut is to be seen in a less completely developed embryo 14 mm. long. From now on the common duct is attached to the left side of the gut which is faced somewhat cranialward, due to its growth anteriorly and to the right. The length of the common bile-duct before its division varies. In a 35 mm. embryo modelled the common duct is quite long and has a distinct turn shortly before it entered the gut. Here again the pancreatic duct opens into the common duct. There has been a continual change of position of the two ducts from the earliest stage to the fully developed one. In an embryo 13 mm. long a distinct pancreatic duct is seen ventral to the common duct. In the further development with the gradual rotation of the liver to the right there has been a change in position of the common duct until in the 35 mm. embryo it lies to the left of the pancreatic which is the condition found in the adult (fig. 44).

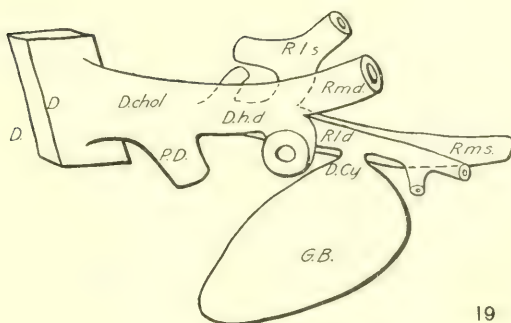
c. Development of the major hepatic ducts. The earliest indication of the hepatic ducts was pointed out in the description of the formation of the liver. In a model of an embryo approximately 5 mm. long, as previously stated, the cavity of the early hepatic anlage extends far laterally. On either side the cavity is constricted dorso-ventrally. From the drawings shown by

² In the further study of the pancreas it was found that this duct was attached by means of a small tubule to the left side of the ventral duct of the pancreas. The epithelial lining resembled that of the gall-bladder, for which this duct was mistaken at first. It might very well be a pancreatic bladder. The pancreatic duct in this embryo was to the right of the enlarged duct.

Choronshitzky it is probable his lateral cylindrical extensions are the early hepatic ducts. In *Amblystoma* these lateral extensions form only the lateral rami of the hepatic ducts. The medial rami are shown in the model of an embryo about 7 mm.



18



19

Fig. 18 Graphic reconstruction (lateral view) of the biliary apparatus of an *Amblystoma* embryo 13 mm. long. $\times 100$. *D.*, duodenum; *D.chol.*, ductus choledochus; *D.h.d.*, right hepatic duct; *D.cy.*, cystic duct; *G.b.*, gall bladder; *R.l.d.*, right lateral ramus; *R.l.s.*, left lateral ramus; *R.m.d.*, right medial ramus; *R.m.s.*, left medial ramus; *P.D.*, pancreatic duct.

Fig. 19 Graphic reconstruction (lateral view) of the biliary apparatus of an embryo approximately 13.5 mm. long. $\times 100$. For abbreviations see figure 18.

long (fig. 36). On the right side in this model there is a lateral extension of the hepatic lumen. A longitudinal ridge in the floor of this side shows a beginning constriction into lateral and medial rami. The medial ramus is more dorsal in position and appears as a swelling on the outer surface. On the left side there

is a wide cavity. On the external surface there is a slight dorso-ventral furrow, an indication of the beginning division into lateral and medial rami.

In an embryo approximately 9 mm. long the right side shows a more marked lateral ramus. The medial still somewhat dorsal ramus is to be seen (fig. 37). Here the left side shows a marked dorso-medial and a ventro-lateral prolongation. The outer surface of both sides of the organ shows many projections, the beginning of tubules from these main rami. The cystic duct though slightly to the right shows more of a constriction from that side. The anterior lip of the cystic evagination also is very prominent.

The rami are formed from the early hepatic ducts by a caudalward constriction and by elongation. Mitotic figures are to be seen at this stage but are more numerous in later ones. As is true of fishes (Scammon '13) there is a relative and actual reduction in the size of these ducts.

In another 9 mm. embryo the development of the ducts is seen to have progressed rapidly (fig. 38). Numerous mitotic figures are to be seen in different sections indicating a rapid growth of the ducts. There are distinct right and left hepatic ducts which show a marked growth. There is a medial longitudinal ridge in the ventral wall of the ductus choledochus indicating a caudalward progressing constriction and division (fig. 38). The cystic duct (*D. cy.*) is distinctly differentiated and attached to the right of the beginning constriction in the common duct. It extends ventrally and somewhat towards the right. The right hepatic duct as seen in figure 38, and in a figure of a model of the cavity of ducts (fig. 20) is divided into a lateral and a dorso-medial ramus. The lateral ramus is further divided into lateral dorsal and medial ventral branches. The left ramus also has medial and lateral divisions.

In embryos from 10 to 12 mm. in length, the trabeculae present a confusing network. The epithelium of both the hepatic ducts and trabeculae are heavily laden with yolk-granules, and that of the ducts is not yet differentiated into a distinct columnar type. However, the right and left hepatic ducts are clear. In

an 11 mm. embryo the right duct is distinctly divided into lateral and medial rami. A short cystic duct is attached to the caudal end of the lateral ramus and on its ventral side. In an embryo somewhat less than 13 mm. long the same arrangement of a short common duct and right and left hepatic ducts is present. The right duct is divided into the medial and lateral rami. The cystic duct here projects somewhat to the left and dorsalward connecting as before with the right lateral ramus.

In a graphic reconstruction of a 13 mm. embryo (fig. 18) the right hepatic duct is divided into lateral and dorso-medial rami. The short cystic duct extends upward and opens into the

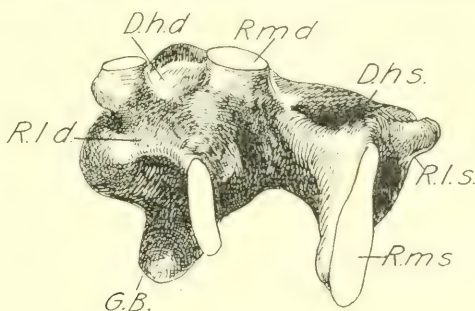


Fig. 20 Anterior view of a reconstruction of the lumina of hepatic ducts and gall-bladder of a 9 mm. embryo. $\times 100$. *D.h.d.*, right hepatic duct; *D.h.s.*, left hepatic duct; *G.b.*, gall bladder; *R.l.d.*, right lateral ramus; *R.l.s.*, left lateral ramus; *R.m.d.*, right medial ramus; *R.m.s.*, left medial ramus.

right lateral ramus. A short lateral branch is the only other division of the right lateral ramus. The dorso-medial branch shortly breaks up into trabeculae. The left duct is also divided into rami. The differentiation of hepatic ducts from trabeculae is now clearer as the epithelium of the former is columnar in type.

In figure 19 from an embryo less than 1 mm. longer than the above, the formation of ducts is seen to have continued. The right hepatic duct is divided into lateral and medial rami, each of which is further divided into dorsal and ventral branches. The same holds true in a general way for the left hepatic duct and its divisions.

In the ventral view of the model of a 14 mm. embryo (fig. 39) the relation of pancreatic duct to the common duct is shown. The short thick common duct divides into right and left hepatic ducts (figs. 39 and 40). They lie in almost the same horizontal plane and are of about the same diameter, but the right is the shorter, dividing almost immediately into its lateral and medial rami. In a 13.5 mm. embryo (fig. 41) the right hepatic duct is of larger diameter than the left. In a 15 mm. embryo the common duct is very short (fig. 42). The right and left hepatic ducts here are very long as compared with those in other embryos. The left duct has come to lie in a more ventral plane due to the shifting of the whole posterior part of the liver and gall-bladder to the right. The same is true to a greater extent for the left ducts in the 20 and 35 mm. embryos (figs. 43 and 44). In a 20 mm. embryo the right hepatic duct is the shorter as it is in a 35 mm. embryo. In a 35 mm. stage the left hepatic duct is almost ventral to the right. The same holds true for a 45 mm. embryo. In the adult, however, the left duct is again more lateral to the right, but still somewhat more ventral.

d. Development of the minor hepatic ducts. Right lateral ramus. The right hepatic duct in a 14 mm. stage is divided into lateral and medial rami and the right lateral ramus is subdivided into lateral and medial branches (fig. 39). The short cystic duct is attached to the lateral branch. The medial branch (fig. 40) gives off several tubules in an oblique dorso-ventral plane. In a 13.5 mm. embryo the right lateral ramus is quite ventral to the medial one (fig. 41). As in the earlier stage, it is divided into lateral and medial branches. The cystic duct which is now directed almost horizontally, is attached to the right side of the lateral branch. The anterior portion of the lateral branch anastomoses with a duct from the right medial ramus. In a 15 mm. embryo (fig. 42) the right lateral ramus is shorter than in the preceding specimen. The right hepatic duct is, however, longer so that the cystic duct is attached to the lateral branch farther from the gut. The lateral branch here divides into dorsal and ventral branches. In a 20 mm. embryo the right lateral ramus is very short (fig. 43). In position it is now somewhat

dorsal to the right medial ramus. It soon breaks up into dorso-lateral and ventro-medial branches. Both of these branches are very long. At the attachment of the cystic duct to the lateral branch there is a further division of the lateral again into medial and lateral radicles. The medial branch has anastomoses with the right medial hepatic ramus. Its further division is in a dorso-ventral plane. In a 35 mm. embryo the right lateral ramus divides into dorsal and ventral branches (fig. 44). There is another division of the dorsal branch and the cystic duct is attached to the dorsal one of this last division. Frequent anastomoses are formed between the tubules of the dorsal and ventral branches, and between those of the dorsal branch and those from the right medial hepatic ramus, as also of the left medial ramus.

Right medial ramus. The right medial hepatic ramus of a 14 mm. embryo as shown by model is very simple (fig. 39). It joins with the left medial ramus, the further division of this common ramus is into right and left branches. The division of the medial ramus is very short and its lateral and medial branches long. Caudally directed tubules are given off from the lateral branch. The medial branch here is connected with the right lateral ramus. The medial branch divides dorso-ventrally into tubules. In a 15 mm. embryo (fig. 42) the medial hepatic ramus is again very simple. It is short and divides into lateral and medial branches of which the latter is given off almost at right angles and from its anterior surface are given off several tubules. The medial hepatic ramus in a 20 mm. embryo as in a 14 mm. one is joined with the left medial ramus (fig. 43). The resulting common ramus divides into a right dorsal (*R. Br.*) and a left ventral branch (*L. Br.*). From the right dorsal branch, dorso-lateral tubules are given off some of which are directed caudally. In a 35 mm. embryo (fig. 44) the right medial ramus is on the same horizontal plane as the right lateral. Its divisions are also into dorsal and ventral branches. Many anastomoses are found between the tubules of this ramus. Tubules from this ramus join those from the right lateral and from the left medial ramus.

Left medial ramus. The left medial ramus is joined to the right medial in a 14 mm. embryo (fig. 39). In a 13.5 mm. embryo the left medial is long and divides into dorsal and ventral branches (fig. 41). Also in a 15 mm. embryo is the left medial ramus quite long (fig. 42). It divides into medial and lateral branches both of which have dorsal and ventral tubules. The left medial ramus in a 20 mm. embryo (fig. 43) is joined to the right. The left ventral branch of this combined duct divides shortly into dorsal and ventral radicles. In a 35 mm. embryo (fig. 44) the left hepatic ramus is quite long. Its anastomoses with the other rami have been noted. There are also several anastomoses with the left lateral ramus.

Left lateral ramus. In a 14 mm. embryo the left lateral ramus is very simple, dividing into medial and lateral branches (fig. 40). The left lateral ramus in the next stage shows further development and growth (fig. 41). In a 15 mm. embryo this ramus has lateral branches given off at quite an angle (fig. 42). It is shorter than the left medial ramus and divides into medial and lateral branches, the latter sending tubules far out to the side. The left lateral ramus in a 20 mm. stage is given off nearly at right angles to the left hepatic duct (fig. 43). It divides into dorso-medial and ventro-lateral branches. In this case the lateral branch is the longer. Several tubules go out laterally almost at right angles and from these tubules hepatic columns go posteriorly as well as anteriorly. In a 35 mm. embryo (fig. 44) the left lateral ramus forms quite a network of ducts. The ventral branch makes an arch forward and is then divided into anterior and posterior branches. In an embryo 45 mm. long the main hepatic ducts are more nearly on the same horizontal plane. Of these ducts the left hepatic has extended farther to the left.

e. Development of the gall-bladder and cystic duct. The gall-bladder appears somewhat later than the liver as noted by Hammar ('97). It arises as a median ventral outpouching caudal to or in the posterior end of the hepatic anlage. Choronzitzky has figured the anlage of the bladder in a median, sagittal section. The structure is shown as a slight depression developing from the gut, at the entrance of the common duct, and

a definite fold is shown between this and the ventrally extending lumen of the hepatic anlage. Greil ('05) showed the gall-bladder in a Bombyinator embryo of 7 mm. length caudal to the hepatic tissue but more closely connected with the liver than with the yolk-mass behind it. In an embryo approximately 7 mm. long, which is undoubtedly an earlier stage in *Amblystoma* (fig. 3) there is no distinct fold between the gall-bladder and liver-anlage. Only a slight median depression of the floor at the posterior end of the hepatic diverticulum is present. No difference is shown by ordinary stains in the epithelium lining this early cystic evagination and that of the liver. Not until later does the epithelium change into the low cuboidal type characteristic of the adult gall-bladder.

A little later the depression in the floor of the hepatic diverticulum is considerably increased (fig. 36). The position of the gall-bladder with reference to the opening of the hepatic anlage has not changed. In a model of liver and gall-bladder of a 9 mm. embryo (fig. 37) the evagination is quite deep. There is a distinct lateral constriction of the dorsal opening of the gall-bladder and distinct anterior and posterior lips to the evagination, indicating the formation of a cystic duct (fig. 4). There is also a deep furrow anterior to the evagination separating the gall-bladder from the hepatic anlage. The posterior furrow is even more marked. The gall-bladder is, however, still very wide laterally.

In another embryo approximately 9 mm. long the gall-bladder has a long cranio-caudal diameter. The furrow marking off the gall-bladder from the hepatic tissue laterally is distinct. The cystic duct is short and of large diameter and it, as well as the gall-bladder, lies to the right of the midline. The cystic duct projects upward and to the left (fig. 21).

A section of the gall-bladder of an embryo 11.5 mm. long shows there has been a continual shifting to the right (fig. 6). The cystic duct has become longer but is still of wide diameter. It projects more to the left and upward. The gall-bladder, though embedded between hepatic tissue and caudal yolk-mass, is completely separated from both (fig. 22). In figure 23 is

shown an increased cranio-caudal diameter, although the transverse is still the greater. The cystic duct here projects more to the left, still somewhat dorsally and slightly backward. The cranio-caudal diameter increases rapidly from now on, and the position of the cystic duct would indicate that there is a more rapid caudal growth. Figure 23 shows the model of a gall-

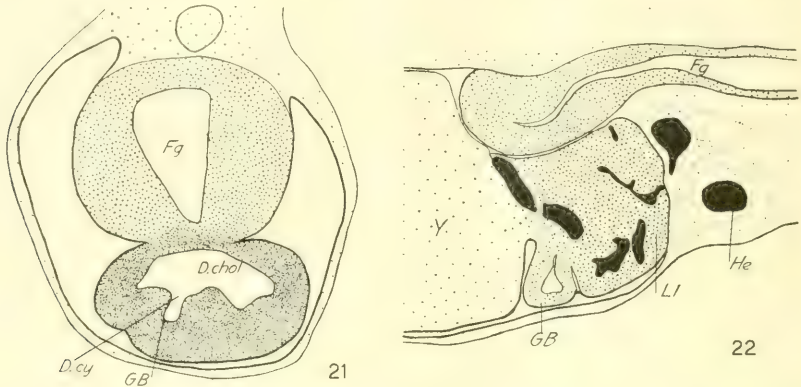


Fig. 21 Transverse section of an *Amblystoma* embryo 9 mm. long, taken in the region of the gall bladder. $\times 30$. *D.chol.*, ductus choledochus; *F.g.*, foregut; *D.cy.*, cystic duct; *G.b.*, gall-bladder.

Fig. 22 Sagittal section of an embryo 12.5 mm. long. $\times 30$. *F.g.*, foregut; *G.b.*, gall-bladder; *Li.*, liver.

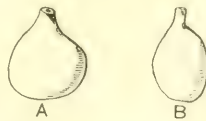


Fig. 23 Drawing of a model of the gall-bladder of an *Amblystoma* 14 mm. long. A, anterior view; B, left lateral view. $\times 40$.

bladder of an embryo almost 14 mm. long. The cystic duct attached near the anterior end, projects to the left and dorsally.

In two graphic reconstructions of embryos 13 and 13.5 mm. in length respectively (figs. 18 and 19), the gall-bladder is attached by a short and constricted cystic duct to a radicle of the right hepatic duct. In figure 18 the cystic duct leads from the anterior dorsal end of the gall-bladder to the left, caudally and

somewhat dorsally, the gall-bladder being distinctly to the right of the midline. In figure 19 the larger of these two embryos the cystic duct is not quite at the anterior end, but the cranio-caudal length of the gall-bladder is distinctly greater. The general direction of the cystic duct is the same. The gall-bladder is relatively as far caudally here as the one shown in figure 18. From the connection of the cystic duct to the gall-bladder, it appears that there has been a marked growth cranialward.

In an embryo 14 mm. (fig. 39) long the gall-bladder has decidedly increased in its cranio-caudal diameter. In transverse section it is almost circular. The cystic duct is of very small diameter as compared with its earlier size. It projects now somewhat upward but almost directly to the left, due to the increased lateral shifting of the liver and the gall-bladder. In this embryo the cystic duct is attached to the extreme anterior dorsal end of the gall-bladder.

Figure 41 is of a model of a 13.5 mm. embryo. In this the general shape of the gall-bladder is the same as of the one just described, except that there is a slight increase in the vertical diameter (fig. 9). The cystic duct, however, is not attached at the extreme anterior end but to the left upper side. It extends towards the left as before but is now almost horizontal.

In a 15 mm. embryo the attachment of cystic duct to the gall-bladder is further caudalward than the previous one (fig. 42). This seems to mark the limit in its caudal attachment for all sizes examined. It would be difficult to say whether this shifting in attachment of the duct to the gall-bladder were due to a difference in the antero-posterior growth of the gall-bladder or to the rapidity of differentiation and growth of hepatic ducts. The cystic duct in this embryo extends toward the left, but now slightly ventrally, which can be taken as evidence of continued rotation to the right and dorsalward of the entire biliary apparatus (fig. 10).

Marshall ('93) has described the gall-bladder of amphibians developing as a lateral outgrowth from the bile ducts. From

its position at this stage one could easily be led to such a conclusion.

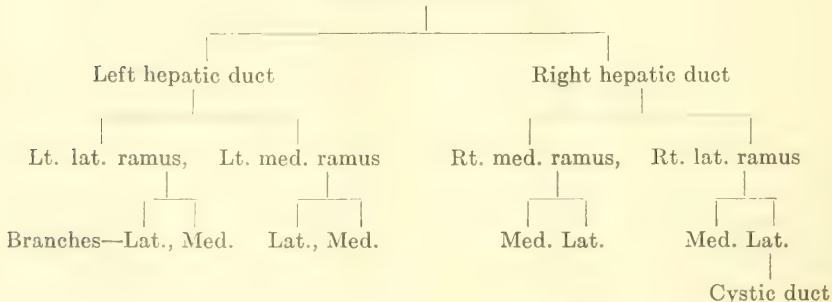
The gall-bladder of a 20 mm. embryo shows a very distinct dorso-ventral increase in diameter (fig. 11). With this there has been a marked cranio-caudal lengthening (fig. 43). The relative size of the gall-bladder is now greater. As before indicated, the cystic duct is here again nearer the anterior end, it extends towards the left and now distinctly ventralward (fig. 11). A right lateral and slightly ventral view of the gall-bladder is shown in figure 43.

In a 35 mm. embryo (fig. 44) the vertical diameter of the gall-bladder has greatly increased. The cystic duct is now in the left anterior ventral end extending ventrally and to the left. In a 45 mm. embryo the gall-bladder has the same general shape as in the preceding, and the cystic duct has not changed in position (fig. 12).

In a graphic reconstruction of the biliary apparatus of a 10 cm. *Amblystoma* the cystic duct extends to the left, somewhat ventrally and anteriorly (fig. 16). The gall-bladder is pear shaped (fig. 13) with its large, blind end projecting slightly dorsally and to the right but mainly caudalward.

f. Summary of the development of the biliary apparatus. In summarising the development of the hepatic ducts a table of the ducts as found in the various models will bring out more clearly their relations to the main duct. Table 3a to 3d shows the principal variations found in the hepatic and cystic ducts.

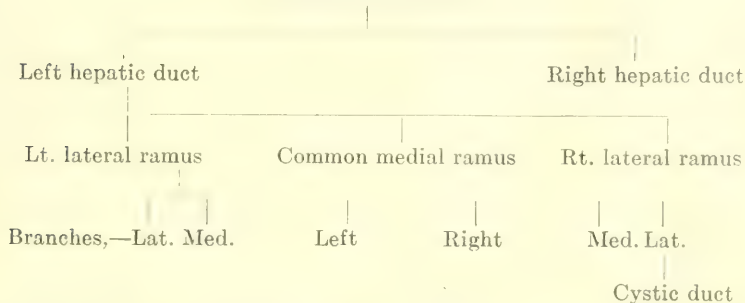
TABLE 3a
Ductus choledochus



Or, in case of anastomoses of the medial rami, as was found in two embryos of 14 and 20 mm. length and two older *Amblystoma* of 7 and 10 cm. length respectively, the following table is given:

TABLE 3b

Ductus choledochus



The cystic duct is attached as here shown:

TABLE 3c

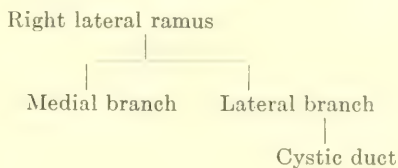
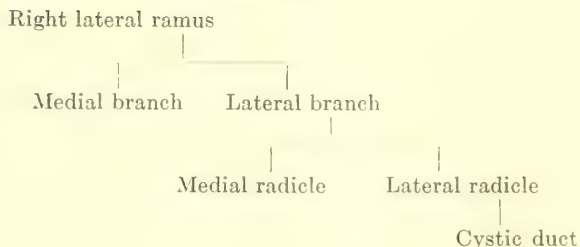


TABLE 3d



or as found in a 35 mm. embryo and one of the larger *Amblystoma*.

From these tables it will be seen that sometimes the right and left medial rami are joined. The division of the common medial ramus is into right and left branches. In their position

and final division these branches are the same as the right medial and left medial rami. As will be seen in figures of the different models, the smaller embryos did not have all of the divisions and subdivisions marked in the tables. In figure 39, for instance, the right branch of the common medial ramus shows no further division, the left branch only one. Further division of both is seen in the 20 mm. stage (fig. 43). The division here, however, is more into dorsal and ventral radicles, due to the more marked lateralward shifting of the liver and the ducts. The extreme of this lateral shifting is seen in figure 44, where the left hepatic duct is almost ventral to the right. The left lateral ramus in a 45 mm. embryo does not hold such a ventral position with reference to the left medial.

There seems to be no definite rule in regard to the anastomosing of ducts. In a 35 mm. embryo they are the most frequent and here apparently because the ducts were crowded so close together. That the right and left medial rami sometimes join and form one duct is seen in the models of a 14 and a 20 mm. embryo, also in the graphic reconstruction of a 7 cm. and 10 cm. *Amblystoma*. It would seem this fusion of the ducts is quite probably due to crowding.

The definite position of the hepatic ducts with reference to the portal vein is seen for all embryos (figs. 8 to 12). The same relation is also found in the adult. As a rule there is a branching of the hepatic ducts corresponding to the division of this vessel. In the developing embryo the ducts are found usually to the right of and ventral to the portal vein.

From the usual description of the biliary apparatus in the frog it would seem that there is a fairly close correlation in the main features between these two amphibians. The figures of Ecker, Wiedersheim and others show a gall-bladder connected to a right hepatic duct. There is also a left hepatic duct, the two uniting in the pancreas and forming a ductus choledochus which, as usually described, is joined by the pancreatic duct. In no case in *Amblystoma* were two cystic ducts found as is shown for the frog. The division into rami in the frog as far as the ducts have been figured, seems to be somewhat different from that

found in *Amblystoma*. The more marked divisions of the liver into several lobes may partially explain this. The duct-system as found in *Necturus* is quite different. Kingsbury here described three hepatic ducts opening into the gut. These anastomosed with each other and two were joined by the ventral pancreatic ducts. The third is a duct direct from the gall-bladder which, however, anastomoses with the other hepatic ducts. Grönberg ('94) described three hepatic ducts which unite with the cystic duct and form a ductus choledochus in *Pipa americana*.

Bates ('04) has described the hepatic ducts in *Amblystoma*. According to his description there are four hepatic ducts, two of which join the bile-duct in its course through the pancreas and the other two just as it opens into the intestine. It is possible that the two he found joining the bile-ducts are the right medial and lateral rami, and the other two, the left medial and lateral rami. In that case the ductus choledochus and the right and left hepatic ducts were very short as was found in some of the material used in this work. Or it may be that the two ducts which joined the bile-duct as it opened into the intestine are the two pancreatic ducts which have not fused until just at the ostium of the hepatic duct. The first two ducts then would be the right and the left hepatic ducts. I have never seen the cystic duct (bile-duct as Bates terms it) open directly into the common hepatic duct.

From the models and drawings it will be seen that the gall-bladder at first has a wide dorsal communication just caudal to the hepatic lumen. As this communication constricts there is formed a short large cystic duct extending dorsally into the right hepatic duct. With further growth and division the cystic duct extends more and more to the left until at the 15 mm. stage it is almost horizontal and at the 20 mm. stage projecting ventrally and somewhat anteriorly. Its earliest attachment is to the ventral surface of the common bile-duct, but in the lateral-ward shifting of the whole liver its attachment goes to the left side of a right hepatic radicle. The connection of the cystic duct to the gall-bladder in early stages is to its dorsal surface about midway between cranial and caudal pole. Somewhat

later the connection is nearer the cranial end and usually reaches the extreme anterior end. The cranio-caudal growth of the gall-bladder has kept pace with the lengthening and differentiation of ducts in the 13 to 14 mm. stage. From the relations in a 15 mm. embryo it appears that the gall-bladder has shifted anteriorly. In this case the hepatic ducts have lengthened more than the gall-bladder. At 20 mm., however, there has been a marked increase in cranio-caudal growth of the gall-bladder so that it is almost as long as the ducts.

Beginning about at this stage the cystic duct is again attached nearer the anterior end of the gall-bladder. This may be taken as evidence that the cystic duct really shifts in its attachment to the gall-bladder. This seems to be borne out in some cases by the fact that its attachment to the hepatic ducts is to a division of the lateral branch of the right lateral ramus instead of to the lateral branch proper. In some cases where the lateral branch is quite long the attachment may have remained to it.

Whether the gall-bladder originates from the early hepatic anlage or from the gut has caused much discussion. As said before Piper ('02) thought this a matter of interpretation. The more marked furrow caudal to the gall-bladder might be taken as evidence of its belonging to the hepatic anlage, also the fact that the same type of yolk-laden cells form hepatic tissue and gall-bladder. That it, at least is directly caudal to the hepatic anlage is proven by the early connection of its duct to the common bile-duct.

The connection of the cystic duct probably depends to some extent on the extent of growth and division of the hepatic ducts. It will be remembered that in the earlier stages the cystic duct opens into the common duct, then into the early right hepatic. In the further growth and division of the right hepatic duct the cystic duct becomes attached to one of its radicles. As noted above, the cystic duct opens into the lateral branch of the right lateral ramus in all of the embryos studied except one, which was 35 mm. long.

That there is considerable variation in the relative dorso-ventral position of these main hepatic ducts is to be expected.

However, in general, a study of the models shows a close similarity in their positions. There is a constant rotation of the liver towards the right and with this is a similar one of the hepatic ducts. In this rotation the right ducts come to be more dorsal in position, the left more ventral. The right lateral divisions would thus be dorsal to the right medial and the reverse should be true for the left. In general such an arrangement is found. A variation in the length of the different ducts is present. However, there is quite a definite relation in the total lengths of ducts in the different embryos. In a 15 mm. embryo the common duct is quite short but the greater length of the hepatic ducts compensates for this reduction. In a 35 mm. embryo the common duct is long, the hepatic ducts and their radicles divide shortly.

III. THE DEVELOPMENT OF THE PANCREAS AND PANCREATIC DUCTS

1. *Literature*

The literature concerning the development of the amphibian pancreas like that regarding the liver is divisible into two periods, and Goette's work ('75) may again be said to mark the beginning of the newer one. The older observers mainly considered the pancreas as a part of the liver, or a modified lobe of that organ.

A list of the investigators describing the development of the pancreas will be found included in the tabular classification of the literature on the development of the liver (table 2).

Goette ('75) in his studies on the development of the Bombinator recognized three distinct pancreatic anlagen, two ventral and one dorsal. The dorsal one he described as placed just caudal to the gastroduodenal loop. The two symmetrical ventral anlagen develop from the primitive hepatic duct. Of these the right grows dorsalward to join the ventral growing dorsal anlage. The right duct changes in position until it opens into the left side of the hepatic duct. The united right and left duct then separates from the common bile-duct. Apparently

Goette considered the left outpouching as a rudimentary one. Later the dorsal duct disappears, thus leaving but one permanent pancreatic duct.

Balfour ('81) and Hertwig ('88) described a dorsal outpouching of the gut wall caudal to the level of the common bile-duct.

The development of the pancreas in both Urodela and Anura was described by Goeppert ('91). In both he found as Goette had described, one dorsal and two symmetrical ventral outpouchings. A constriction of the early dorsal outgrowth forms a duct, while folds and ridges developing on the blind end give rise to the glandular tissue. The right and left ventral ducts unite on the right side of the common bile-duct. However, he found two pancreatic ducts opening into the common bile-duct in an adult, also three pancreatic ducts that fused immediately before opening into the common bile-duct. The numerous dorsal ducts which he found in the adult urodeles he explained are of secondary origin. He, too, found only one pancreatic duct persisting in adult Anura.

Marshall ('93) stated that in the frog the pancreas developed as a pair of hollow outgrowths caudal to the liver. Later the ducts shift and open into the bile-duct instead of, as at first, into the intestine.

Minot ('93) in his text book of embryology, mentioned that in urodeles the dorsal duct persists, and in Anura only the ventral duct.

Weyse ('95) and Stöhr ('95) both agreed with the description of Goette and Goeppert. Stöhr was especially interested in the dorsal pancreatic anlage. He did not find a double dorsal pancreas as had v. Kupffer ('92) in one of the ganoids. He believed that the caudal dorsal pancreatic anlage described by v. Kupffer is part of the hindgut. Brachet ('96) reviewed the descriptions of earlier investigators.

Woit ('97), a student of v. Kupffer, and probably influenced by his views, in his work on the development of the spleen stated that the dorsal pancreas gives rise to the spleen as well as to a part of the adult pancreas in urodeles. He described two persisting ducts in urodeles.

Gianelli ('99) described an "intrahepatic pancreas" in Triton, the tubules of which are in intimate relation with the liver-tubules, and it is stated by him are continuous with them.

Reuter ('00) made mention also of the early appearance of the dorsal and ventral pancreas. Both arise from the anterior part of the midgut region (Anfangsdarm) from the yolk-cells. The pancreas, as well as the liver, is found in the gastro-duodenal loop as soon as this is formed, and both are at first to the right and dorsal to the intestinal spiral.

Choronshtzky ('00) described in *Necturus* and the frog two lateral outpouchings from the early hepatic duct. These two lateral outpouchings form the ventral ducts, later they unite posterior (ventral) to the common duct. He described two ducts in the adult urodeles.

Gianelli ('02) described three distinct pancreatic anlagen in Triton. The dorsal anlage develops first. The ventral anlagen develop from the posterior end of the hepatic outpouching as two masses of vitelline cells into which the lumen of the hepatic evagination later extends. The right and left outpouchings both fuse with the dorsal pancreas. The ventral pancreatic duct formed from both pancreatic anlagen opens into the hepatic duct. The left pancreas remains in intimate relation with the liver.

Braun ('06) described the early development of the pancreas in *Alytes obstetricans*. He found a dorsal and two ventral pancreatic anlagen, which are first to be recognized as swellings of the yolk-gut wall and by the more numerous nuclei. The ventral anlagen are caudal to the anlage of the hepatic duct. The right ventral pancreas is somewhat more caudal than the left, joins the dorsal pancreatic outpouching and later joins with the left ventral pancreas. The dorsal outpouching loses its connection with the yolk-gut soon after coming in contact with the right ventral pancreas. The cells forming the pancreas are at this time still undifferentiated yolk-cells. Differentiation of the cells and glandular development take place at the same time. The early ventral outpouchings develop into the pancreatic ducts which unite just before opening into the gut to the right of

the hepatic duct. The pancreas in the adult lies in the gastroduodenal loop.

Eycleshymer and Wilson ('10) described the two ventral anlagen as dorso-lateral to the ductus choledochus. These appear some time after the single dorsal anlage, and union with the dorsal pancreas does not take place until the embryo reaches a length of about 29 mm. They found that the dorsal duct opens into the duodenum just caudal to the stomach, and also mentioned two ventral ducts.

It is generally agreed by those who have described the duct system in the adult urodeles that at least two ducts persist. Hyrtl ('65) by means of injection in adult *Cryptobranchus* found two pancreatic ducts, one of which joined the hepatic duct. Oppel ('89) in *Proteus* has described an anterior and a posterior set of ducts, the latter emptying into the ductus choledochus. Kingsbury ('94) found one anterior duct opening just behind the pylorus, and two caudal ducts which open separately into the ductus choledochus. One pancreatic duct has been described as persisting in adult *Anura*. Bates ('04) stated that the pancreatic ducts join the hepatic ducts as they pass through the pancreas.

2. Early development of the pancreas and pancreatic ducts

As stated in the description of the liver, the pancreas develops later than that organ. A well marked dorsal pancreas is to be found in embryos 8 mm. long. A mass of cells in the dorsal wall of the enteron is separated by a distinct transverse furrow from the anlage of the stomach in front and from the yolk-mass behind. Mitotic figures are to be found in this mass of cells.

In embryos about 9 mm. long there are three pancreatic anlagen, two ventral and one dorsal, as has been described for other amphibia. The two ventral anlagen appear as evaginations posterior to the hepatic outpouching and caudal to the ventral-lying gall-bladder. The evaginations of the pancreas on the ventral wall of the gut extend in a longitudinal direction for

some distance. Anteriorly there is quite a distinct furrow between the liver and the pancreas. A model of this stage (fig. 24) shows the outpouchings of the pancreas and of the gall-bladder and liver anteriorly. Posteriorly there is no sharp demarcation of the pancreas from the yolk-mass and gut. A model of the lumina of the pancreatic evaginations and of the gall-bladder and hepatic ducts makes the position of the different parts with reference to the antero-posterior plane more clear (fig. 25). At this stage the pancreatic anlagen are caudal to the gall-bladder which is directly anterior to the right pancreatic evagination.

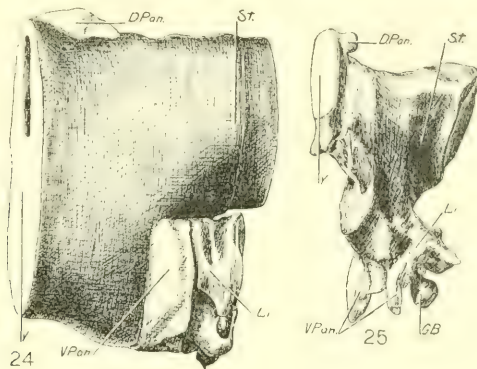


Fig. 24 Lateral view of a reconstruction of the pancreatic anlagen of a 9 mm. embryo. $\times 40$. *D.pan.*, dorsal pancreas; *Li.*, liver; *St.*, stomach; *V.pan.*, ventral pancreas; *Y*, yolk-gut.

Fig. 25 Lateral view of a reconstruction of the lumina of the gut and pancreatic anlagen. $\times 40$. *G.b.*, gall-bladder; other abbreviations, as in figure 24.

The pancreatic evaginations extend farther ventrally than do the hepatic ducts and the gall-bladder.

The right and left pancreatic anlagen are separated anteriorly by a slight ventral furrow. Caudally the two evaginations are apparently fused as the area between them is bulged ventrally. The evidence of a division into two evaginations is much more clear in a view of the model of the lumina of the pancreatic anlagen (fig. 25). This is also brought out clearly by the figure of a section taken about 80μ caudal to the gall-bladder (fig. 26). In this figure one sees the two very definite evaginations, and

that they are separated as far as the transverse width of the gut will permit. On the lateral side there is an indefinite furrow at about the level of the dorsal margin of the liver extending caudalward in the wall of the gut and yolk-mass. This marks the upper limit of the ventral pancreatic Anlagen (fig. 24). In

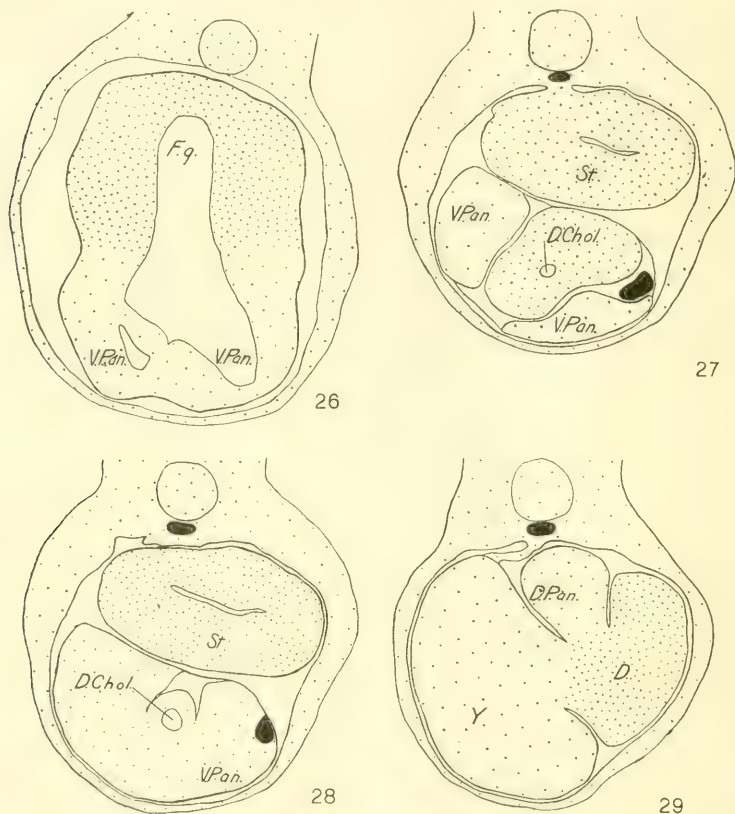


Fig. 26 Drawing of a section through the ventral anlagen of an embryo 9 mm. long. $\times 40$. *F.g.*, foregut; *V.Pan.*, right and left ventral pancreases.

Fig. 27 Drawing of a section through the ductus choledochus of an 11 mm. embryo. $\times 40$. *D.chol.*, ductus choledochus; *St.*, stomach; *V.Pan.*, ventral pancreases.

Fig. 28 Drawing of a section about 80μ anterior to the preceding. $\times 40$. For abbreviations, see figure 27.

Fig. 29 Drawing of a section through the dorsal pancreas of an 11 mm. embryo. $\times 40$. *D.*, duodenum; *D. pan.*, dorsal pancreas; *Y*, yolk gut.

figure 26 the lumina of the two ventro-lateral pancreatic diverticula open widely into a common lumen which connects dorsally with the gut-cavity. Anteriorly at about the caudal end of the gall-bladder this lower common lumen is separated from the lumen of the intestinal anlage as shown by the model of the lumina of the ducts (fig. 25) as well as by the figure of a model of the hepatic ducts (fig. 20). In the section figured (fig. 26) the lower part of the right evagination is separated from the main lumen by cells. The next section anteriorly shows the left lumen also cut off. The pancreatic lumina thus very early extend somewhat forward.

The dorsal pancreatic anlage, as shown by both models (figs. 24 and 25), is median and further caudalward than the ventral. As seen in figure 24 it seems to be an elevated portion of the wall of yolk gut. The anterior and posterior furrows separating the anlage from the stomach and gut are not so prominent in this specimen. A model of the lumen shows it to be directed forward (fig. 25). Stöhr's statement that there are no evidences of double dorsal pancreatic anlagen in any stages as has been described in the ganoids by v. Kupffer is true also for *Amblystoma*. The dorsal anlage at this time is short in its cranio-caudal diameter. It is, however, further developed than the ventral anlagen. Its ventral margin is limited by a slight groove at the anterior end. Caudally this groove is not present. Figure 24 as well as figure 25 shows that there has been quite an increase in the dorso-ventral diameter of the intestine. That the ventral part is becoming constricted from the dorsal is shown by both models and was pointed out in the description of the development of the liver. From the anterior end of the ventral part of the gut is the hepatic outpouching, caudal to this and on the right side the gall-bladder, and still farther posteriorly the ventral pancreatic anlagen. Caudal to these evaginations again the gut lumen takes a more dorsal position.

In 10 mm. embryos the dorsal pancreas is much more prominent. The furrow separating it from the stomach is quite deep. Also the caudal furrow is well marked. Mitotic figures are more numerous than before. The cells lining the evagination

are columnar in type but still contain considerable yolk. The lumen extends a very short distance forward.

In an 11 mm. embryo there has been considerable further development of the midgut region. The stomach has differentiated to some extent. It has flattened dorso-ventrally, and its posterior end is constricted and shifted to the left. The duodenum extends ventrally to the left and has an anteriorly directed portion which forms, with the stomach, the gastroduodenal loop. At its anterior end which Brachet ('95) has termed the 'seconde courbure' in *Axolotl* the duodenum turns to the right and is continuous with the caudal-extending yolk-mass. Here at the cranial end is the ventral pancreas. The pancreatic area appears at this stage as a narrow zone of the gut marked off by furrows, anteriorly from the hepatic area and posteriorly from the duodenum and yolk (fig. 30). The pancreatic ducts are short and extend ventro-laterally from either side of the common duct. These ducts are caudal to that part of the common bile-duct which gives off towards the right ventral side the cystic duct and anterior and somewhat dorsally two lateral hepatic ducts. The groove separating the anterior end of the pancreas from the hepatic tissue is well marked. The gall-bladder extends downward and to the right of the midline between pancreas and liver. A drawing of a section near the anterior end of the duodenal loop shows what appears to be a constricted forward projection of the gut (fig. 27). Somewhat anteriorly the cells lining this constricted gut are of a tall columnar type heavily laden with yolk as are the cells lining the duodenum (fig. 28). This is the caudal end of the common duct. Posterior to the section figured one can see the two lateral parts of the pancreas distinctly separated from the constricted anterior end of the gut where the common duct is attached (fig. 27). About fifteen sections of 10μ anterior to this are the ventro-laterally projecting pancreatic ducts. The pancreas has grown both anterior and posterior to the ducts, but the greater growth has been forward (120μ caudalward and 140μ anterior). The left pancreas grows anteriorly sending a small projection to the left of the gall-bladder. A model of the pancreas of this stage

shows it as a cap placed over the anterior end of the gut, distinctly separated from it by a groove and closely united to the liver which lies in front of it. The right side shows only slight indication of its later dorsal and caudal growth (fig. 30).

The dorsal pancreas forms an irregular elongated mass to the right of the gastro-duodenal loop, but extends somewhat caudal to it. To the right of the pancreas and ventrally lies the large yolk-mass (fig. 30). The dorsal pancreas is now relatively and actually nearer the ventral pancreas than in earlier stages. There is as yet little evidence of any ventral growth of the anterior

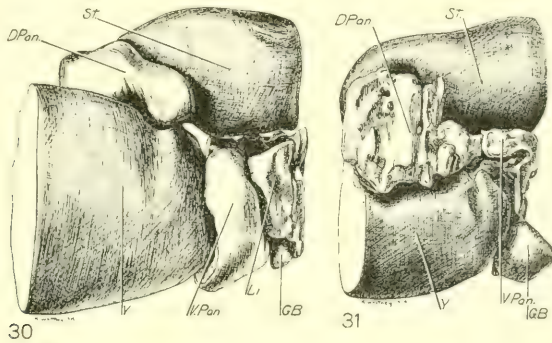


Fig. 30 Lateral view of a model of the pancreatic anlagen of an 11 mm. embryo. $\times 40$. *D.pan.*, dorsal pancreas; *G.b.*, gall-bladder; *Li.*, liver; *St.*, stomach; *V.pan.*, ventral pancreas; *Y*, yolk gut.

Fig. 31 Lateral view of a model of the pancreas of a 13 mm. embryo. $\times 30$. *D.pan.*, dorsal pancreas; *G.B.*, gall-bladder; *St.*, stomach; *V.pan.*, ventral pancreas; *Y*, yolk-gut.

end. The duct as in the younger stages lies mainly in the cranial part of the mass forming the dorsal pancreas. It extends to the right and dorsalward and is nothing more than a constricted part of the evagination. As seen in figure 29, it is attached to the archenteron near the large yolk-gut. The segment of the gut to which the dorsal pancreatic duct is attached is the caudal end of the duodenal loop in Brachet's ('95) 'ascending limb' which posteriorly is completely constricted from the ventral yolk-mass. Its attachment here, then as is shown by later stages, is to the dorsal wall of the duodenum.

The development of the duodenum and the gastro-duodenal loop has been described by Goette and others who described the changes which bring the opening of the dorsal pancreatic duct nearer to the pylorus than the ventral ducts. Goeppert ('91, p. 113) stated concerning this: "—so erhält mann in den Schnitten die dorsale Anlage später als die ventralen Anlagen. Wenn mann aber die schräg absteigende Richtung des vorderen Schenkels der Gastroduodenalschlinge berücksichtigt, sieht mann leicht dass das dorsale Pankreas trotzdem einem noch etwas vor der Mündung des Leberstieles gelegenen Theil der Darmwand angehört."

Brachet ('95) has described the position of the digestive tract in young axolotl. The duodenum continuing caudally from the stomach he has termed the first descending limb; the caudal turn, the 'premiere courbure;' then ascending limb, 'seconde courbure,' and second descending limb.

The dorsal and ventral pancreaticanlagen at this stage are composed of masses of cells still containing many yolk-granules. The ducts of both appear as constricted portions of the out-pouching connecting them with the duodenum and the common bile-duct.

During the 12 and 13 mm. stages the dorsal pancreas comes in contact with the ventral one. The dorsal pancreas forms an irregular mass lying to the right of the duodenum and dorsal to the caudal yolk-mass. A small part extends anteriorly and somewhat ventrally and comes in contact with the right pancreas. In a 13 mm. embryo the two masses are fused (fig. 31). The acini of the two actually fuse as is shown by figure 32. The ventral pancreas is the smaller. A small part of the left ventral pancreas lies along the left side of the gall-bladder and anterior to the duodenum (fig. 45). The hepatic ducts extend through the ventral pancreas anteriorly to the liver but have no connection with the former. The right ventral pancreas in figure 31 has not grown dorsalward to any extent to join the dorsal pancreas. The dorsal pancreas in this case has grown ventrally and to the right to join the ventral pancreas.

The dorsal duct is now well developed. It extends dorsally and slightly to the right from the right dorsal side of the duo-

denum near its caudal turn. The duct divides shortly sending out branches in all directions.

The ventral ducts in a model of a 13 mm. embryo come off laterally from the hepatic ducts and immediately divide into smaller rami. As a rule the ventral ducts at this stage fuse into one tube ventral to the common duct. The ducts may join the ventral wall of the common duct or the anterior end of the gut, where the common bile-duct opens into the duodenum (fig. 45).

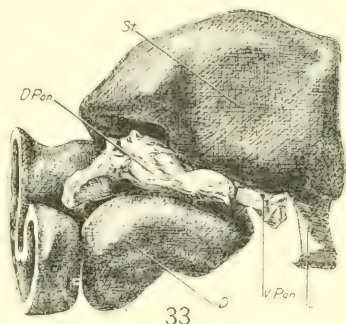
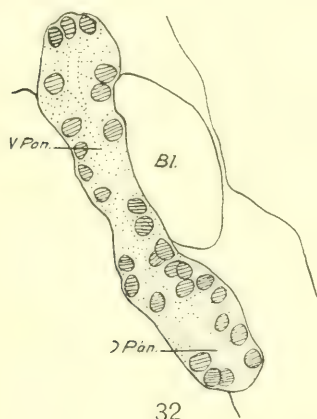


Fig. 32 Drawing of a section showing the united acini of the dorsal and right ventral pancreas. $\times 180$. *D.pan.*, from dorsal pancreas; *V.pan.*, from ventral pancreas; *Bl.*, blood vessel.

Fig. 33 Lateral view of a model of the pancreas of a 15 mm. embryo. $\times 30$. *D.pan.*, dorsal pancreas; *D.*, duodenum; *Li.*, liver; *St.*, stomach; *V.pan.*, ventral pancreas.

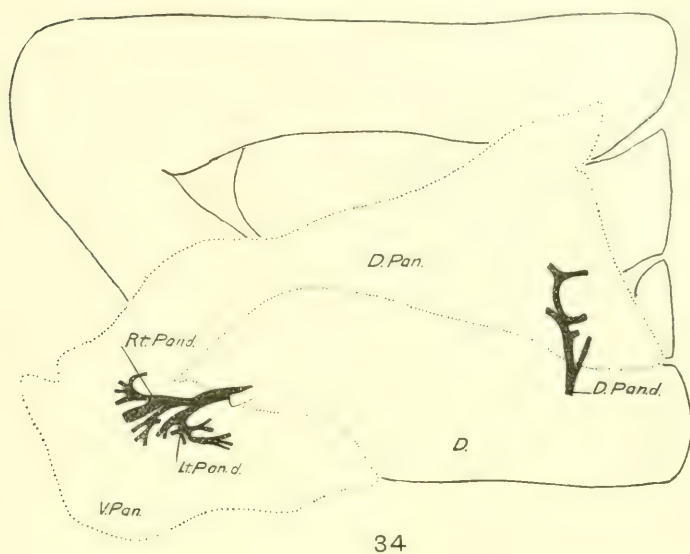
In a 15 mm. embryo as well as in later stages the dorsal pancreas forms the larger part of the whole organ. It joins the right ventral pancreas by a neck of tissue, which is larger than in the preceding stages (fig. 33). The ventral mass is crescentic in transsection and lies just below the stomach, and anterior and somewhat dorsal to the anterior end of the duodenum (fig. 45). A part of the ventral pancreas extends anteriorly along the left side of the gall-bladder. The pancreatic duct opens into the gut ventrally and slightly to the left of the common bile-duct (figs.

42 and 46). The pancreatic duct directly divides into two branches, a right and left, which end shortly. The dorsal duct has the same position as in the preceding stage.

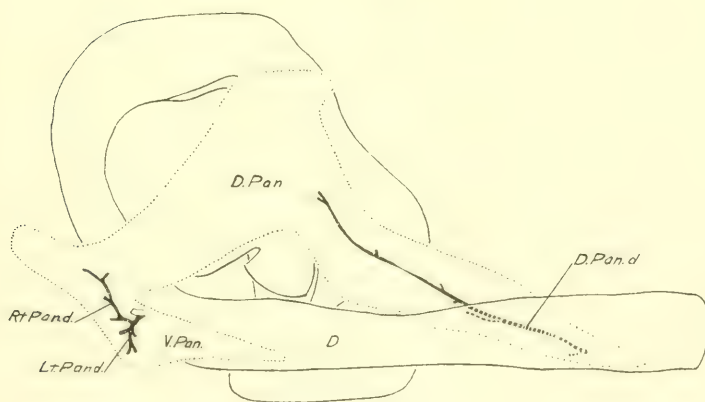
In the following stages there is an increase in the size of the whole pancreas. The dorsal portion comes to lie more and more dorsal to the duodenum and along the right wall of the stomach, while the ventral portion increases in size anteriorly, in front of the anterior duodenal loop.

A description of the parts in a 35 mm. embryo is given as typical of the further development of the pancreas. In this stage the anterior part of the pancreas lies ventral to the gall-bladder and is embedded in the peripheral part of the liver (fig. 7, *B*). This part later probably forms the intrahepatic portion of the pancreas described by Gianelli ('99). Somewhat caudally it is considerably larger in section and separates the liver into dorsal and ventral portions (figs. 11 and 12). In this region just caudal to the gall-bladder the hepatic and ventral pancreatic ducts lie embedded in the pancreas. The pancreas is rather prismatic in cross section, its medial side lying along the right side of the stomach, its lateral dorsal side bounded by the duodenum. Slightly anterior to the ostium of the common duct the pancreas is divided into two masses, one lying ventral and somewhat to the left of the duodenum, the other to the left of the duodenum between it and the stomach (fig. 7, *C*). This latter mass joins the anteriorly directed portion of the dorsal pancreas. The remainder of the ventral pancreas now lies to the lower right side of the stomach, with a part projecting caudalward along the ventral wall of the duodenum (fig. 34). The dorsal pancreas is caudal to the ventral and takes a more dorsal position, until it comes to lie above the duodenum which has migrated downward. In cross section the dorsal pancreas extends from the lower right side of the stomach almost to its dorsal margin. About half a centimeter from its caudal end the pancreas forms a very small mass, triangular in section, dorsal to the duodenum.

The ducts of the ventral pancreas of a 35 mm. embryo are shown in a graphic reconstruction in figure 34. The ventral



34



35

Fig. 34 Graphic reconstruction of the pancreas and pancreatic ducts of a 35 mm. embryo. $\times 20$. D., duodenum; D.pan.d., dorsal pancreatic duct; D.pan., dorsal pancreas; Lt.pan.d., left ventral pancreatic duct; Rt.pan.d., right ventral pancreatic duct; V.pan., ventral pancreas.

Fig. 35 Graphic reconstruction of the pancreas and pancreatic ducts of a 20 cm. Amblystoma. $\times 5$. For abbreviations, see figure 34.

duct arises with the common bile-duct from a fold in the lower left wall of the duodenum (fig. 7, *C*). It shortly separates from the common bile-duct and extends anteriorly along its right side. It is somewhat ventral to the hepatic duct and divides into dorsal and ventral ducts. The ventral duct shortly sends off branches caudalward to the left ventral portion and extends forward in the ventral anterior part (fig. 34). The dorsal of the two ducts divides several times into dorsal and ventral rami. Branches from the dorsal duct extend caudalward and to the right into the anteriorly directed portion of the dorsal pancreas. The dorsal pancreatic duct is given off from the upper left side of the duodenum near the caudal end of the pancreas and extends almost directly upward sending off several short branches anteriorly and posteriorly.

3. Description of the adult pancreas

In a 15 cm. *Amblystoma* the anterior end of the pancreas, which is somewhat triangular in section, is embedded in the liver to the right of the stomach. It lies along the upper concave border of the liver with the portal vein and has a small free surface lined with peritoneum. Caudal to this the pancreas lies to the left of the duodenum, between it and the stomach and enlarges in a dorso-ventral direction (fig. 35). Now it passes along the left wall of the duodenum but extends both dorsally and ventrally to it, the ventral portion being between the duodenum and stomach. About 5 mm. by sections from its anterior end, the pancreas divides into two masses, one along the left ventral surface of the duodenum and the other dorsal to it. The ventral mass remains in about the same position, and extends caudalward almost half the length of the body of the gland. At the caudal end of the ventral portion of the pancreas the dorsal mass which has shifted somewhat to the right and lies above other loops of the intestine as well as the duodenum, is divided into two or three irregularly-shaped lobes, one of which is dorsal to the duodenum and directly to the right of the stomach. This part of the pancreas extends a considerable distance cau-

dalward. As the duodenum shifts downward and to the left with relation to the stomach, this portion of the pancreas lies more and more to the right of the duodenum and for a considerable distance it lies ventral to the stomach. The pancreas extends caudalward almost to the gastro-duodenal loop.

A short distance from the caudal end of the pancreas the dorsal pancreatic duct is connected to the right side of the duodenum (fig. 35). This duct extends forward almost to the point where the dorsal pancreas divides into several parts and gives off small branches as it comes to lie more and more in the dorsal part of the gland. The duct of the ventral pancreas joins the common bile-duct (fig. 35) where it opens into the left side of the duodenum or beside it. The pancreatic duct is ventral to the common bile-duct and almost immediately divides into right and left ducts. The left duct is the more ventral and soon divides into branches which turn ventral and caudally (fig. 35). The right duct extends anteriorly and upward and sends some branches into that part of the dorsal pancreas which joins the ventral.

4. Discussion

As has been found in other amphibia, the pancreas in *Amblystoma* is developed from three anlagen, two ventral and one median dorsal. As in other forms the dorsal develops earlier and, as Stöhr and others have stated, from only one evagination. This is found just caudal to the anlage of the stomach. In none of the embryos studied is there evidence of an outpouching toward the left as Goeppert ('91) described in another form. Greil ('05), however, in young *Bombinator* embryos reconstructed right and left dorsal pancreatic outpouchings. Older embryos show that there has been considerable growth of the dorsal pancreas in a cranio-caudal direction with the lengthening of the duodenum, and a division of the anterior end into several processes, one of which joins the ventral pancreas.

In the very earliest stages the ventral anlagen are caudal to the gall-bladder. Goeppert ('91) and Chronshitzky ('00) have described these anlagen as lateral to the cystic evagination

in the forms studied by them. Greil ('05) reconstructed the pancreatic anlagen as well as the branchial pouches of 7 and 7.5 mm. Bombinator embryos and showed the ventral pancreatic evaginations as dorso-ventral to the attachment of the hepatic outpouching and described them as lateral to the hepatic anlage.

In *Amblystoma* the two ventral pancreases unite very early along their medial sides. Braun ('06) has described the union of the dorsal and the right ventral pancreas as occurring before the union of the right and the left ventral anlagen and Greil stated that the right ventral pancreas has united with the right dorsal in 7.5 mm. embryos. The appearance of considerable pancreatic tissue from the left side of the ventral anlage would indicate that there is growth from this evagination. Goette apparently thought this outpouching was rudimentary. The presence of a left duct in some 12-13 mm. embryos indicates that there is growth from the left side. Later stages show that there is more growth on the right side.

Although the lumen of the dorsal pancreatic anlage at first extends anteriorly, the duct later extends upward and in the adult again forward. Short lateral branches extend into the surrounding pancreas. The ventral ducts fuse to form a single one which divides into a right and left pancreatic duct. These again divide into smaller rami, of which some from the right side extend into the portion uniting with the dorsal pancreas. The dorsal and ventral ducts, however, always remain separate.

It is clear that in *Amblystoma* there is no complex pancreatic duct system as Oppel ('90) has observed in *Proteus*. In possessing a single dorsal duct *Amblystoma* resembles the *Necturus* as described by Kingsbury ('94). The posterior set of ducts emptying into the ductus choledochus as described by Oppel is quite different from the single ventral duct in *Amblystoma*. Nor does this conform to Kingsbury's description of two posterior pancreatic ducts each of which open into hepatic ducts. I cannot confirm Bates' ('04) observations concerning the several pancreatic ducts which he stated opened into the hepatic ducts within the pancreas. A table of the ducts as they have been described in various urodeles may be of interest.

As is well known the duct of the dorsal pancreas does not persist in Anura. However, Goeppert ('91) and Vogt and Yung ('94) have described several pancreatic ducts in the ventral pancreas some of which joined the ductus choledochus or hepatic duct. It is seen from the table that there is considerable variation in the pancreatic duct-system of the urodeles. The complex system of some forms may be due, as Goeppert suggested, to a later union of the lesser pancreatic ducts with the duodenum or common duct.

TABLE 4
Table of the pancreatic ducts in the various urodeles

| <i>Form</i> | <i>Author</i> | <i>Dorsal pancreas</i> | <i>Ventral pancreas</i> |
|--------------------------|---------------------|------------------------|---|
| Cryptobranchus japonicus | Hyrtl ('65) | 1 (?) | 1, joining with ductus choledochus |
| Salamandra perspicillata | } Wiedersheim ('75) | 1 (?) | 1, joining the two hepatic ducts |
| Geotriton fuseus | | | |
| Proteus anguineus | Oppel ('89) | 10, 33 | 9, 11 forming network with ductus choledochus |
| Menobranchus lateralis | Goeppert ('91) | | 2, joining ductus choledochus |
| Salamandra maculata | } Goeppert ('91) | 1 | { 1, joining ductus choledochus |
| Salamandra atra | | | |
| Triton alpestris | } Goeppert ('91) | 2 | { 3, joining ductus choledochus |
| Triton taeniatus | | | |
| Cryptobranchus japonicus | Goeppert ('91) | 6 | 1, opening near ductus choledochus |
| Necturus maculatus | Kingsbury ('94) | 1 | 2, joining separate hepatic ducts |
| Triton | Gianelli ('02) | 1 | 1, joining hepaticocystic duct |
| Amblystoma | Bates ('04) | — | Several—joining various hepatic ducts |
| Amblystoma | Baumgartner ('15) | 1 | 1, may or may not join ductus choledochus |

The glandular portions of the ventral and dorsal pancreases fuse as is clearly shown in figure 33. This fusion of the glandular parts takes place immediately after the two parts come in contact. The union of the two parts is at first only a narrow neck of tissue, which remains small even in adults.

In a 35 mm. embryo and in smaller ones attention was called to a small part of the ventral pancreas lying below the gall-bladder and separating the liver into upper and lower parts.

The peripheral portion of the liver grows more rapidly and surrounds this part on the outer side. It then has a peritoneal surface only on the medial upper side. This corresponds to Gianelli's intrahepatic portion of the pancreas. However, as stated by Goeppert, the pancreatic and hepatic tissues are always clearly separate. Goeppert ('91) has given a description

of the relations and lobes of the pancreas. The pancreas in *Amblystoma* resembles that in those forms which he described in having a ventral part or lobe caudal to the liver and in intimate relation with it, and a dorsal or caudal part. Kingsbury described five more or less distinct parts. The following table shows a correlation of the lobes of the pancreas which various investigators have described with those of *Amblystoma*.

TABLE 5

Table of the parts of the adult pancreas in Amphibia

| | | | | | |
|--|---------------------------------------|--------------------------------------|---|----------------------------|---------------------------------|
| Proteus..... Oppel ('89)..... | Vordere Theil | Mittlere Theil | Hintere Theil | | |
| Salamandra, Rana, etc..... Goeppert ('91).. | Dorsal oder vordere Theil | Ventral oder hintere Theil | Hinterste Theil | | |
| Necturus..... Kingsbury ('94) | Lobe along in- testine | Central part near gall bladder | Lobe along dorsal wall of liver | Lobe along splenic vein | Lobe along mes- enteric vein |
| Triton..... Gianelli ('02) .. | Corpo, estre- mità posteri- ore | Estremità craniale | Pancreas intra- epatico | | |
| Alytes obstetri- canus..... Reuter ('06).... | Kopf | Wurzel | | | |
| Amblystoma. . | Dorsal portion | Ventral por- tion | Portion along dorsal con- cave border of liver | | |

Goeppert mentioned that there were usually several other prolongations of pancreatic tissue. This is also true for *Amblystoma*, particularly from the anterior end of the dorsal portion were there several prolongations. It is to be remembered that 'vordere' is used by Oppel and Goeppert to express proximity to the oral end of the intestinal loop and not in the ordinary topographical sense.

IV. GENERAL SUMMARY

1. The liver begins as a median ventral projection of the lumen of the gut, then as an anterior outpouching from this lumen.

2. There is a later shifting of the posterior part of the liver to the right and dorsally, due to crowding of the stomach and development of the duodenum on the left.

3. A later growth on the left side results in an adult organ with right and left parts, the right side always remaining more dorsal on the lateral side of the stomach.

4. The ductus choledochus develops as the early anteriorly directed lumen from the gut.

5. The right and left hepatic ducts develop as divisions of the ductus choledochus and by division and growth form the hepatic rami and branches.

6. The gall-bladder begins as a median ventral outpouching of the posterior part of the liver-anlage. It is first widest laterally, then becomes larger in its cranio-caudal diameter, then its dorso-ventral and finally its longer axis is nearly transverse.

7. There is an early right lateral shifting of the gall-bladder as of the liver, due probably to the same causes. Along with this there is a constant shifting of direction of the cystic duct in keeping with the dorsalward migration of the gall-bladder.

8. The cystic duct is early closed off with the right hepatic and due to the caudalward growth and division of the hepatic duct is finally attached to the lateral branch of this duct.

9. The ventral pancreatic anlagen are ventro-lateral evaginations of the gut caudal to the cystic anlage. The dorsal pancreas—a single median dorsal evagination—forms the larger portion of the early pancreas, later it is a narrow lobe dorsal to the duodenum.

10. The ventral pancreatic ducts are constrictions of the two ventral pancreatic anlagen. Later these unite and form a single ventral pancreatic duct which opens into the common bile-duct or into the intestine at the side of the common bile-duct. The dorsal duct remains a single stem with short lateral branches.

11. There are two main parts or lobes in the adult *Amblystoma* pancreas, a dorsal and a ventral, with one or more lesser projections from these. An anterior extension of the ventral lobe is constant.

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PLATE 1

EXPLANATION OF FIGURES

36 Median view of right and left halves of a reconstruction of the liver of an *Amblystoma* embryo 7 mm. long. $\times 70$.

37 Median view of right and left parts of a reconstruction of the liver of an embryo 9 mm. long. $\times 70$.

38 Posterior view of a reconstruction of the liver of an embryo 9 mm. long. $\times 100$.

d., early anlage of duct

D. chol., ductus choledochus

D. cy., cystic duct

D. m., dorso-medial duct

D. h. d., right hepatic duct

D. h. s., left hepatic duct

G. B., gall-bladder

Li., liver

Lt. Rt., left and right parts of hepatic anlage

v. l., ventro-medial duct

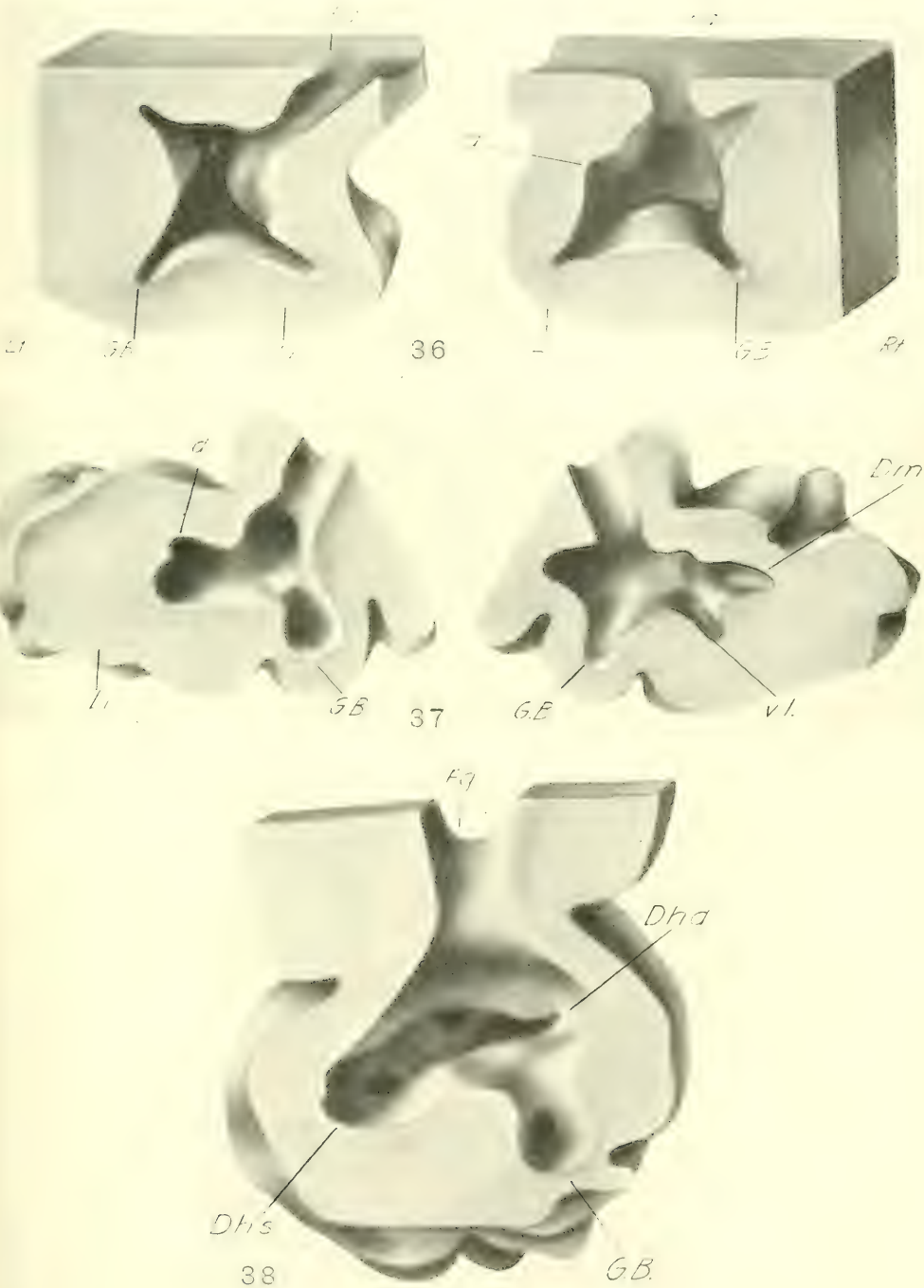


PLATE 2

EXPLANATION OF FIGURES

39 Ventral view of a reconstruction of the hepatic ducts and gall-bladder of an *Amblystoma* embryo 14 mm. long. $\times 100$.

40 Dorsal view of the same reconstruction. $\times 100$.

41 Ventral view of a reconstruction of the hepatic ducts and gall-bladder of an embryo 13.5 mm. long. $\times 100$.

42 Dorsal view of a reconstruction of the hepatic ducts and gall-bladder of an embryo 15 mm. long. $\times 100$.

43 Right ventral view of a reconstruction of the hepatic ducts and gall-bladder of an embryo 20 mm. long. $\times 100$.

| | |
|--|---|
| <i>D.</i> , duodenum | <i>M.R.l.d.</i> , medial branch right lateral ramus |
| <i>D.chol.</i> , ductus choledochus | |
| <i>D.cy.</i> , cystic duct | <i>M.R.l.s.</i> , medial branch left lateral ramus |
| <i>D.h.d.</i> , right hepatic duct | <i>M.R.m.d.</i> , medial branch right medial ramus |
| <i>D.h.s.</i> , left hepatic duct | |
| <i>D.P.</i> , pancreatic duct | <i>M.R.m.s.</i> , medial branch left medial ramus |
| <i>g.b.</i> , gall-bladder | |
| <i>L.Br.</i> , left branch of common ramus | <i>R.Br.</i> , right branch of common ramus |
| <i>L.R.l.d.</i> , lateral branch right lateral ramus | <i>R.l.s.</i> , left lateral ramus |
| <i>L.R.l.s.</i> , lateral branch left lateral ramus | <i>R.l.d.</i> , right lateral ramus |
| <i>L.R.m.d.</i> , lateral branch right medial ramus | <i>R.m.s.</i> , left medial ramus |
| <i>L.R.m.s.</i> , lateral branch left medial ramus | <i>R.m.d.</i> , right medial ramus |
| | <i>Z.</i> , extra duct in 13.5 mm. <i>Amblystoma</i> embryo |

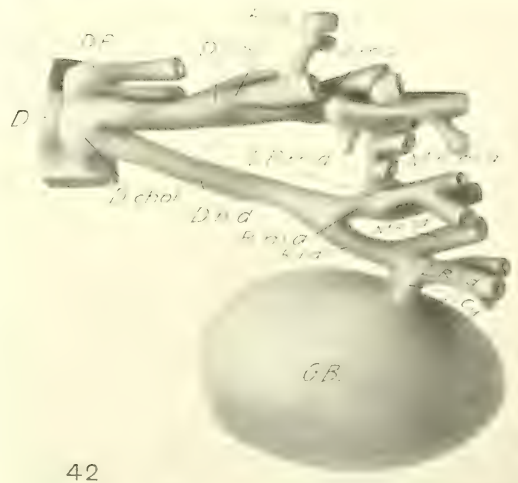
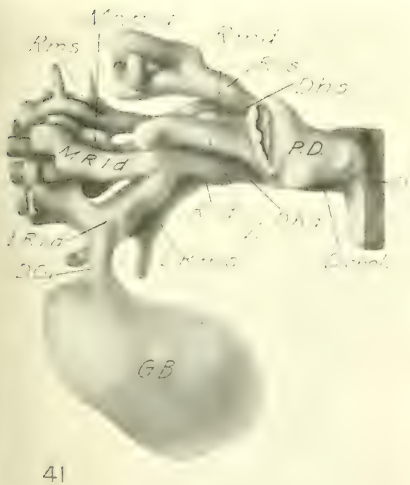
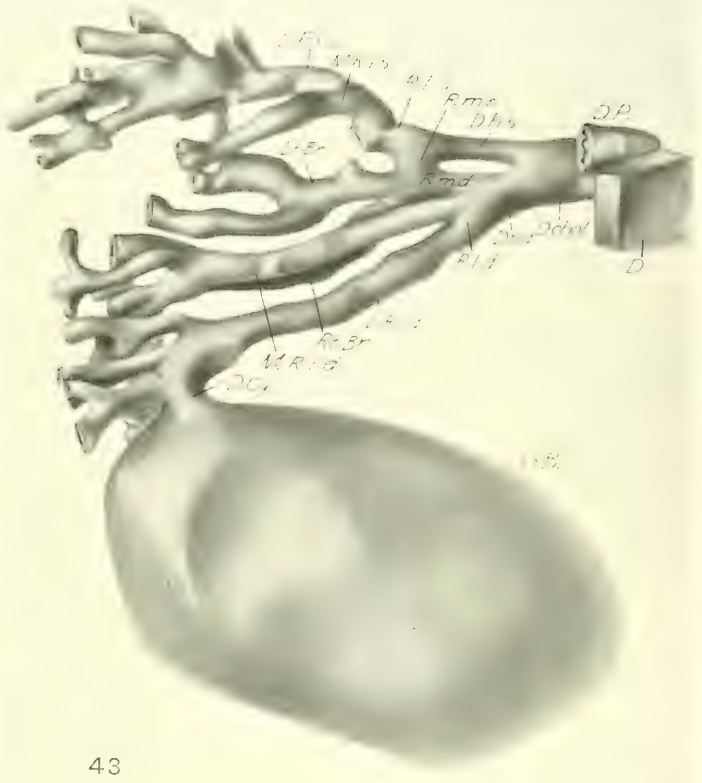
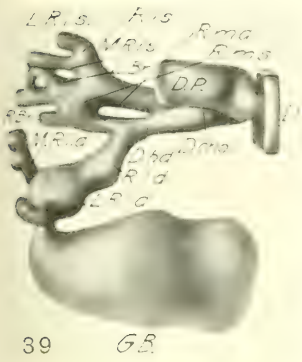


PLATE 3

EXPLANATION OF FIGURES

44 Right ventral view of a reconstruction of the hepatic ducts and gall-bladder of an *Amblystoma* embryo 35 mm. long. $\times 70$.

| | |
|--|---|
| <i>D.</i> , duodenum | <i>L.R.m.s.</i> , lateral branch left medial ramus |
| <i>D.chol.</i> , ductus choledochus | <i>M.R.l.d.</i> , medial branch right lateral ramus |
| <i>D.cy.</i> , cystic duct | <i>M.R.l.s.</i> , medial branch left lateral ramus |
| <i>D.h.d.</i> , right hepatic duct | <i>M.R.m.d.</i> , medial branch right medial ramus |
| <i>D.h.s.</i> , left hepatic duct | <i>M.R.m.s.</i> , medial branch left medial ramus |
| <i>D.P.</i> , pancreatic duct | <i>R.Br.</i> , right branch of common ramus |
| <i>g.b.</i> , gall-bladder | <i>R.l.d.</i> , right lateral ramus |
| <i>L.Br.</i> , left branch of common ramus | <i>R.l.s.</i> , left lateral ramus |
| <i>L.R.l.d.</i> , lateral branch right lateral ramus | <i>R.m.d.</i> , right medial ramus |
| <i>L.R.l.s.</i> , lateral branch left lateral ramus | <i>R.m.s.</i> , left medial ramus |
| <i>L.R.m.d.</i> , lateral branch right medial ramus | |

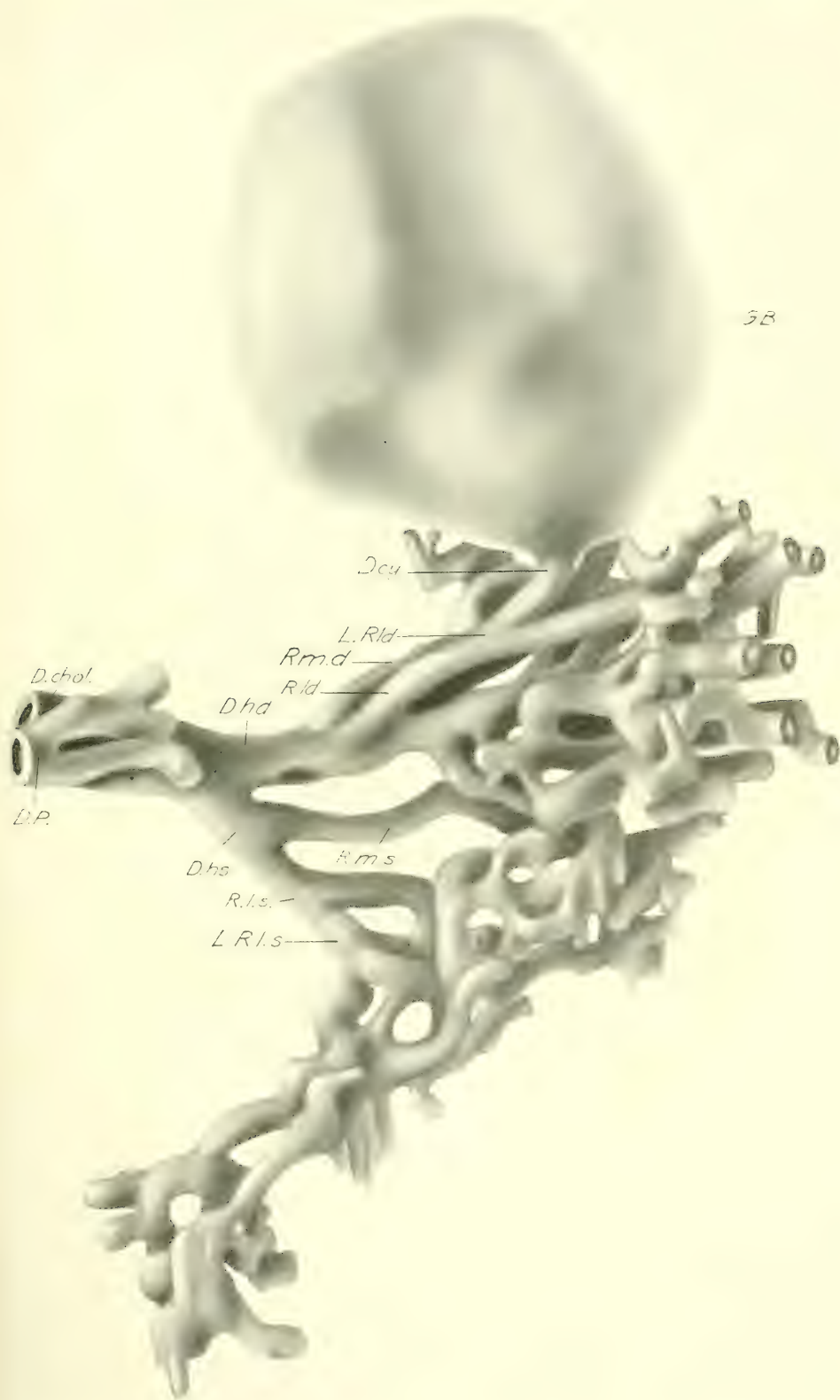


PLATE 4

EXPLANATION OF FIGURES

45 Anterior view of the pancreas and ventral pancreatic ducts of a 13 mm. embryo. $\times 60$.

46 Anterior view of the pancreas and ducts of a 15 mm. embryo. $\times 60$.

D., duodenum

D.pan., dorsal pancreas

G.B., gall-bladder

Lt.pan.d., left ventral pancreatic duct

Rt.pan.d., right ventral pancreatic duct

St., stomach

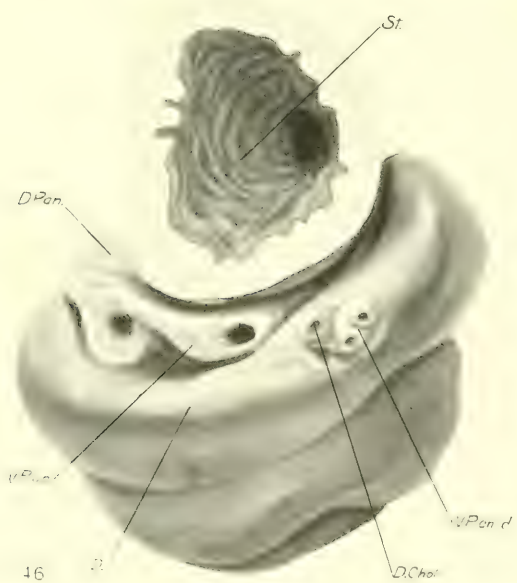
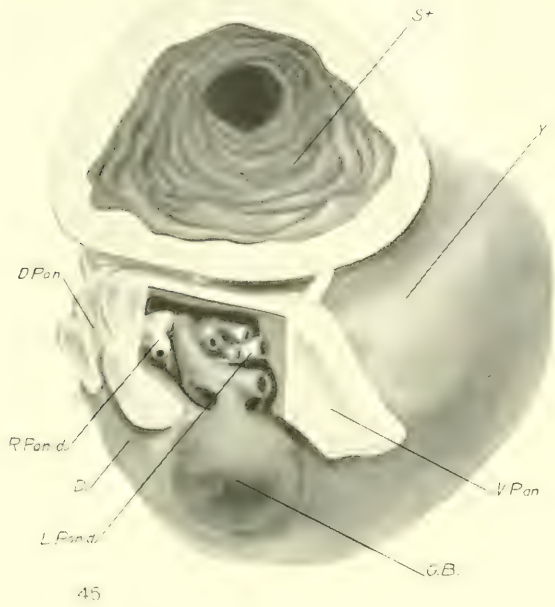
V.pan., ventral pancreas

Y, yolk-gut

D.chol., ductus choledochus

V.pan.d., ventral pancreatic ducts.

For other abbreviations, see figure 45.



THE MICROSCOPIC STRUCTURE OF THE YOLK-SAC OF THE PIG EMBRYO, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE ERYTHROCYTES

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THIRTY-FIVE FIGURES (TWO PLATES)

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I. INTRODUCTION

The chief purpose in view in this study of the yolk-sac of the pig embryo was the acquisition of further data regarding the earliest stages in blood cell origin and development in mammals. The yolk-sac was believed to be the most favorable material for the search for evidence concerning the disputed relationship between mesenchyma, primitive endothelium and haemoblasts. The pig embryo was selected for study on account of its ready availability. It was hoped that information could be contrib-

uted to the following debated questions in haemopoiesis: 1) Does the angioblast bear any direct genetic relationship to the entoderm? 2) Does the yolk-sac mesothelium produce haemoblasts? 3) Does the mesenchyma differentiate in part into endothelium? 4) Do haemoblasts arise directly from mesenchyma? 5) Do haemoblasts differentiate from endothelium? 6) What is the origin and function of the giant cells of the yolk-sac? The first question involves a careful consideration of the structure of the entoderm; which in turn raises the question: 7) What is the function of the entoderm in yolk-sacs which contain little or no yolk?

A preliminary report of this study appeared in the Proceedings of the thirty-first session of the American Association of Anatomists (*Anat. Rec.*, 9: 1, '15, pp. 92-97). In the present paper more extensive observations, with illustrations, are recorded. Moreover, a further study of the entoderm compels a reinterpretation of the cytoplasmic filaments of these cells; my earlier conclusion that they are mitochondrial in nature no longer seems warranted.

A portion of this investigation was done at the Marine Biological Laboratory, Woods Hole, Massachusetts during the summer of '14. I take this opportunity to gratefully acknowledge my indebtedness to the institution for the privileges of a research room.

II. MATERIAL AND METHODS

The material consists of pig embryos ranging in length from 5 to 25 mm. Zenker's and Helly's fluids were used for fixation. The stains employed were the Giemsa blood stain, and the haematoxylin and eosin combination. Sacs of stages within the limits specified differ essentially only with respect of relative abundance of the various types of early blood cells. The 10 to 15 mm. stages were soon discovered to be most favorable for this study, since here was included in the same sections both earliest and later stages in haemopoiesis. Haemopoietic phenomena seem to be at their height in the yolk-sac of the pig embryo at about the 10 mm. stage of development.

III. DESCRIPTIVE

a. The entoderm

It seems preferable to begin the description of the histology of the yolk-sac with the entodermal constituent of its wall.

In the 5 mm. stage the entodermal cells are cuboidal, and arranged in a single layer; there is as yet no trace either of solid or tubular evaginations into the enveloping mesenchyma.

In the 10 mm. stage of development the lining cells are columnar, the taller being about twice the length of the tallest cells in the earlier stage; they are still arranged in a single layer. However, there is great variation in the form of the cells; the predominating type of entodermal cell is columnar, but all transitional forms appear from very low cuboidal to tall columnar cells. At certain points the entoderm invaginates the mesenchyma in the form of short cords and tubules. The 'tubules' are scarcely more than shallow folds, but recall the larger branched tubules of the yolk-sac of human embryos of this length (Meyer (18); Jordan (10).) The condition is probably to be interpreted in terms of a mechanical adjustment on the part of the entoderm to the exiguous confines delimited by the enveloping mesenchyma, or it may perhaps be merely a shrinkage phenomenon.

At the 25 mm. stage of development the entodermal cells appear shorter columnar but are still almost invariably arranged in only a single layer. Occasional small stratified areas occur similar to those characteristic of the human yolk-sac of even much earlier stages, but they are perhaps most correctly interpreted as short stout entodermal buds or cords. At this stage the very sparse enveloping mesenchyma is extensively invaded by very numerous robust solid cords and irregular tubules of entodermal cells. In tangential sections the yolk-sac wall of this stage looks strikingly like reptilian liver tissue.

The cytology of the entoderm is essentially identical for the 5 to 25 mm. stages (figs. 2 and 31). The vesicular nucleus is relatively large and spherical, and is generally placed nearer the basal pole. It contains one or several large, spheroidal,

chromatic nucleoli (fig. 2) and a delicate wide-meshed granular reticulum. Many of the cells are undergoing mitosis. The cell wall appears distinct. But there is no indication of terminal bars nor brush borders, such as have been described for the entodermal cells of the human yolk-sac by Branca (2). In Giemsa-stained preparations the cytoplasm is colored dark blue, the nuclei light blue, and the nucleoli bluish orange or lilac.

The most striking feature of these cells is the presence of a generous amount of delicate filaments (basal filaments; ergastoplasmic filaments) scattered throughout the finely granular basophilic cytoplasm. They are oriented in general parallel to the long axis of the cell. They may be coarser or finer, in length equal to that of the entire cell or much shorter; and they may be apparently homogeneous or segmented (fig. 31). The latter condition would seem to indicate the possibility that they may fragment into secretion granules, but the evidence for this conclusion is not wholly satisfactory. Their probable significance and nature will be discussed in a later section. It may suffice here to state that the cells of the liver (fig. 32) and those of the mesonephric tubules contain apparently identical cytoplasmic threads; and that in no case do they bear any direct relationship to mitochondria, which must have been dissolved by the fixing fluids used.

b. The mesothelium

The outer surface of the yolk-sac wall, like the homologous layer of the splanchnopleure generally, is characterized by a layer of greatly flattened cells each bulging more or less at the point where the nucleus is located. The cytoplasm is delicately reticular like that of the underlying mesenchyma, with which the mesothelial cells are apparently in syncytial continuity (figs. 2 and 4). The nuclei are generally relatively large, oval, vesicular structures, with one or several small irregular net-knots, and a delicate wide-meshed nuclear reticulum (figs. 9 and 20). In their general form, structure and light staining capacity they are practically identical with the nuclei of the mesenchyma and

the endothelium (figs. 13 to 18). In the Giemsa stain the nuclei of these three tissues are similarly colored bluish orange, while the cytoplasm stains a lighter blue. Occasional cells may be seen in mitosis, but there is no clear evidence to indicate that their proliferation products may differentiate into haemoblasts. The proliferation is most probably related only to the extension of the mesothelial covering. Certain cells, however, are more or less rounded, simulating early stages in the formation of haemoblasts from endothelium (fig. 20).

c. The mesenchyma

The mesenchyma is of very variable amount in different portions of the wall (figs. 2, 4, 29 and 30); in certain regions it is so sparse as to be barely discernible between the entoderm and the mesothelium; in other regions it may greatly exceed in width that of the tallest portions of the entoderm. It is a loose-meshed syncytium containing numerous spaces and occasional small blood islands, and larger and smaller blood vessels or sinusoids (figs. 29 and 30). Around certain spaces the mesenchymal cells may become arranged so as to very closely simulate endothelial cells. Indeed it seems impossible to differentiate between such a cell and certain endothelial cells from blood-cell-containing channels. It seems difficult to avoid the conclusion that endothelium is thus differentiated from the mesenchyma, the differentiation depending here as in the case of the structurally apparently identical mesothelium, upon the mechanical factor of pressure (fig. 29). Many of the mesenchymal nuclei are in some phase of mitosis, and occasional nuclei appear to be dividing amitotically.

d. The endothelium

The cells lining blood-cell-containing channels are flattened elements, of fusiform shape in sections. The commonest type of cell contains a vesicular oval nucleus, practically identical with that of the mesenchyma and the mesothelium (figs. 4, 13 and 29); and also the delicate reticular cytoplasmic structure

of the endothelial cells is like that of these cells. Moreover, the endothelial cells appear to be in direct syncytial continuity with the mesenchyma. Many are in mitosis, and occasional nuclei appear to be dividing amitotically. It seems most probable that they are actually mesenchymal cells modified in shape by the pressure of the confined blood stream. Endothelial cells which lie next the entoderm are sharply separated therefrom (figs. 29 and 30). The entodermal cells rest upon a delicate but distinct basement membrane, with which the endothelium is not in structural continuity (fig. 2). The vascular anlagen (angioblast) are at certain points in direct continuity with the mesenchyme, but are sharply demarked from the entoderm (fig. 29). There is no evidence here that the angioblast has any direct genetic relationship to the entoderm; all the available morphologic data are opposed to the idea of such a relationship.

The endothelium includes, however, numerous cells which may be arranged into a complete series connecting the above described endothelial cell with a haemoblast (figs. 4, 13, 14, 15 and 16). The transition steps consist of a progressive rounding up of the nucleus and a gathering of the cytoplasm around it. At the same time the nucleus enlarges and the cytoplasm appears to increase in amount. Moreover the cytoplasm becomes more highly basophilic and appears finely granular. The cell as a whole, of fusiform shape, becomes progressively shorter and finally separates from the endothelial wall either as a short fusiform cell, or frequently as a spherical cell flattened at its proximal pole and drawn out laterally into delicate processes which gradually separate from the vessel wall (figs. 5 and 6). Such cells may even become multinucleated before separation (figs. 8 and 9), and undergo cytoplasmic differentiation, even elaborating haemoglobin, as will be described below. The multinuclear condition appears to be the result of amitotic nuclear division (figs. 9 and 35). The observation of the differentiation of endothelial cells into haemoblasts is of cardinal importance, and will be more fully discussed in a later section.

e. The blood cells

1) *Terminology.* Four distinct types of cells may be recognized: 1) The haemoblasts, or blood mother-cells. These correspond with the primitive 'lymphocytes' of Maximow (16), and the 'mesamoeboid cells' of Minot (19). 2) The erythroblasts, corresponding with the 'ichthyoid' blood cell of Minot, and in part with the 'megaloblast' of Maximow. 3) The normoblasts, corresponding with the 'sauroid' cell of Minot. The last two may be designated inclusively as erythrocytes. 4) The giant cells, both megakaryocytes and polykaryocytes.

The majority of the blood cells can be classified under one or the other of the above heads. However, between typical primitive haemoblasts and erythroblasts, and between the latter and normoblasts, as also between haemoblasts and giant cells, complete series of transition forms occur.

Up to the 15 mm. stage no cell is present that can be certainly identified as a leucocyte. The haemoblasts are structurally very similar to the lymphocytes of the adult, and if they are indeed in part at least, functionally identical, as claimed by Maximow in support of the monophyletic theory of haemopoiesis, they may be properly designated 'lymphocytes.'

2) *Haemoblasts.* This terminology implies that the cell designated 'haemoblast' is the common mother-cell of both leucocytes and erythrocytes. No evidence, besides its very close similarity to a lymphocyte, accrues from this study to indicate that the cell in question is also a leucocyte progenitor. It may be noted, however, that this cell would apparently have to undergo less differentiation in becoming a mononuclear, or even a polymorphonuclear, leucocyte, than in becoming an erythroplastid. Moreover, there is now a very considerable body of embryologic data to show that this cell in certain mammals (rabbit, Maximow (16); birds, Dantschakoff (5); reptiles, Dantschakoff (6), and Jordan and Flippin (14); and selachii and amphibia, Maximow (17)) is indeed the parent cell of both red and white blood corpuscles. Thus while the haemogenic proc-

ess here to be described is purely erythropoietic, the primitive cell is nevertheless properly termed 'haemoblast.'

The haemoblast is in its youngest form a relatively small cell, ranging from about half to approximately the full size of the definitive normoblast, with a larger nucleus and much less cytoplasm (figs. 1, 2 and 3). It has a relatively enormous nucleus, which is enveloped by a narrow shell of cytoplasm generally wider at one point over an area of from less than a quarter to more than a half of the surface (fig. 1 a). The cytoplasm is finely granular and deeply basophilic. The nucleus is vesicular with one or several spheroidal chromatic masses (nucleoli), scattered irregularly through a wide-meshed, delicate, frequently granular reticulum containing larger chromatin granules peripherally on the nuclear membrane. In Giemsa-stained preparations the nucleoli are colored lilac, the nuclear sap bluish pink, the cytoplasm deep blue. The haemoblast may show several blunt pseudopods indicating amoeboid capacity (figs. 2 and 27). The young haemoblasts are more generally peripherally placed in the blood vessels, the later differentiation stages more centrally.

The haemoblasts show a very wide range of size variations and nuclear forms, while at the same time adhering to a very close structural similarity both nuclear and cytoplasmic (figs. 1, 2, 3 and 7). By growth the primitive haemoblast may become very large; this growth may be chiefly nuclear (fig. 34) or chiefly cytoplasmic (fig. 7). It does not seem possible to draw a sharp line between large haemoblasts and certain so-called 'giant cells,' to be described below. Their essential nuclear and cytoplasmic features are very similar.

By division a larger haemoblast gives rise to smaller, structurally identical, haemoblasts. The mode of division may be mitotic, and apparently also amitotic (figs. 3, c, d, and e, and 22). Cytoplasmic division frequently does not directly follow nuclear division, thus giving rise to binucleated cells (fig. 3 d and e). Similarly, tripolar spindles may produce trinucleated cells (fig. 21), or the same may be probably produced also by direct division (figs. 11 and 12). Multinuclear cells are probably simi-

larly formed (figs. 25 and 35). The bi- and multinucleated types will be further described under 'giant cells.'

Haemoblasts have a double source of origin: 1) from the mesenchyma (fig. 30); 2) from the endothelium of the earliest blood vessels (fig. 4). Since this endothelium, however, also originally arose from mesenchyme, the primary, in part indirect source, is the same, namely the original mesenchyma.

The endothelial origin of haemoblasts has already been partially described above under 'endothelium.' It need merely be emphasized here that the evidence on this point seems unequivocal; transition stages are practically innumerable; their abundance is so great as to make it difficult to adhere to a reasonable limit in the selection of illustrations. Possible objections to the interpretation here given to the observations will be considered below. The above description pertains only to intravascular haemopoiesis; the endothelium contributes also, but apparently much more rarely (except in the mesonephric glomeruli of the body of the embryo), extravascular haemoblasts. The continuity of such with the endothelial wall counter-veils the possible objection that these are migrants (fig. 4).

The direct mesenchymal origin of haemoblasts concerns itself with the blood-islands and certain isolated cells separating from the mesenchymal syncytium. Peripherally the blood-islands are in continuity with the mesenchyma, where endothelial cells are differentiated; centrally the cells are haemoblasts in various earlier stages of metamorphosis into erythroblasts; some of these may be binucleated (fig. 29).

The unique and crucial evidence for mesenchymal origin of haemoblasts pertains to certain isolated cells caught in the actual process of differentiation and separation from the syncytium. These are admittedly rare, but the evidence they furnish is of prime importance. It supplies the link in the monophyletic theory of haemogenesis concerning which there has been the greatest scepticism. Figure 30 is an illustration of the clearest case of the condition referred to. Here is shown an area of mesenchyma in which two of the nuclei, as well as their enveloping cytoplasm, have mesenchymal features; the third nucleus

(h) and its enveloping cytoplasm are of typically haemoblast character. A delicate chromatic nuclear bridge still connects the haemoblast nucleus with the mesenchyma nucleus. The significance of this nuclear bridge is uncertain, but it plainly reveals genetic relationship whatever its meaning in terms of type of cell division. Such instances should definitely dispose of the objection that all mesenchymal haemoblasts are migrants from adjacent blood vessels. Haemoblasts are very variable in form, due to the variable number and form of their pseudopods (fig. 27). They must be regarded as capable of extensive amoeboid motility.

It is a matter of sufficient importance to warrant special emphasis at this point, that between typical haemoblasts and typical erythroblasts, next to be described, transition forms exist abundantly (fig. 1 b). The marks of transition pertain both to the nucleus and the cytoplasm. The change is perhaps most marked in the staining capacity of the cytoplasm. This loses its intense basophily, and in Giemsa preparations becomes a much lighter pink or grayish blue. This chemical alteration inheres principally in the elaboration of a small amount of haemoglobin. The cytoplasm shows also faintly a coarse wide-meshed reticulum. And a distinct cell wall is now evident (fig. 1 b), whereas the haemoblast is apparently a naked cell. The nucleus becomes relatively smaller and more chromatic; the nucleoli tend to disappear, and the nuclear reticulum becomes coarser, more granular and more chromatic.

3) *Erythroblasts*. These cells are characterized by their slightly smaller spherical nuclei and an acidophil cytoplasm generous in amount (fig. 1 c). The nuclei generally lack distinct nucleoli but contain a coarsely granular, intensely chromatic, nuclear reticulum. The cytoplasm has frequently a finely granular appearance (fig. 3 f). In Giemsa preparations the nucleus stains blue, the cytoplasm a faint brownish pink. These cells are much more uniform in size than the haemoblasts and are generally mononuclear. They undergo very extensive mitotic proliferation. The transition stages (figs. 1 b and 3 f) between the haemoblast and the erythroblast, characterized by a bluish

pink color in Giemsa preparation, correspond to the 'megalo-blast' described by Maximow in the rabbit.

Occasionally a disintegrating erythroblast may be seen ingested by an endothelial cell (fig. 28). This observation indicates a phagocytic function on the part of the endothelium of the yolk-sac vessels. An alternative interpretation will be discussed below.

4) *Normoblasts*. The normoblasts differ from the erythroblasts in that they have a smaller more compact and chromatic nucleus, and a more acidophilic cytoplasm (figs. 1 d, 2 e and 3 g). These cells are very uniform in size. In this character of size uniformity they differ markedly from the similar cells in certain lower forms, for example, in turtles. They multiply extensively by the indirect method of cell division. In Giemsa preparations the cytoplasm stains a brilliant red, the coarsely granular nucleus a deep blue. The nucleus frequently has an irregular lobed contour. The chromatin is frequently gathered into several large and many smaller clumps, the reticulum being delicate and only slightly chromatic. In preparations fixed in Zenker's fluid, the haemoglobin content has become dissolved, and the cytoplasmic area reveals a coarse wide-meshed reticulum, bounded peripherally by a coarse cell membrane (fig. 3 g). By abstriction of the portion of the cytoplasm containing the excentric nucleus, in the manner described by Emmel (7), the erythrocyte becomes an erythroplastid. These stages in plastid formation are still extremely rare in 10 mm. embryos.

5) *Giant cells*. These cells include a great variety of different forms and sizes. The extremes include: 1) An enormous cell consisting almost wholly of nucleus, the naked cytoplasm constituting a mere shell (figs. 33 and 34). The cytoplasm is basophilic. The vesicular nucleus is generally extensively lobed and contains many large spheroidal and irregular chromatic masses; its nuclear reticulum is wide-meshed, granular, and intensely chromatic. 2) A cell of similarly large size with generally two or three relatively small, spherical, oval or irregular, pale staining, granular nuclei (figs. 23, 24 and 25). The nuclei may contain one or several nucleoli; and the reticulum is more regular,

more delicate, sometimes double (fig. 23) and less deeply chromatic. The cytoplasm is slightly acidophilic. Both nuclear and cytoplasmic features resemble those of the erythroblasts ('megakaryoblasts'). 3) A cell of similar or even larger size with numerous nuclei (as many as eight are common) of various shapes and sizes and differing in structure between the two extremes above described (figs. 11, 12 and 35). The cytoplasm of such a cell is also more or less basophilic.

The origin of giant cells can be definitely traced by means of transition stages to the haemoblasts. Type 1, above described, is simply a giant haemoblast (compare figs. 3 a, 7 and 33). Type 2 is a giant haemoblast with several nuclei (compare figs. 3 a and 11) derived by nuclear amitotic division—occasionally possibly also by nuclear mitosis—unaccompanied by cytoplasmic division. The cytoplasm has entered upon the early stages of differentiation into erythroblast cytoplasm. Type 3 is derived from type 1 by extreme and irregular fission of the single nucleus, accompanied by slight differentiation in the cytoplasm (figs. 12, 25 and 35).

Frequently a typical giant cell with two or even three nuclei may be seen in continuity with the endothelial wall of the blood vessel, and in late stages of separation (figs. 8 and 9). This observation further supports the conclusion of haemoblast derivation of giant cells. There is no evidence in favor of an entodermal origin of giant cells as held by Graf. v. Spee (22) in the case of the human yolk-sac.

A small number of giant cells contain one or several normoblasts. The normoblast periphery may be separated from the enveloping giant cell cytoplasm by a narrow space (fig. 10); or such space may be lacking, in which event the continuity between the two cytoplasmic seems complete (fig. 26). Two possibilities of the origin of these intracellular normoblasts at once suggest themselves: 1) ingestion; 2) differentiation from the nuclei and portions of the surrounding cytoplasm of the giant cell. The fact that endothelial cells (potential haemoblasts) may ingest erythroblasts (fig. 28), as above described, lends much weight to the first suggestion. The further facts, how-

ever—1) that in certain cells with more than one normoblast no haemoblast nucleus remains (fig. 26); 2) that giant cells of the yolk-sac are simply modified haemoblasts whose cytoplasm undergoes a chemical alteration, as indicated by staining reactions, similar to that of haemoblasts in becoming erythroblasts; 3) that no multinucleated giant cells could be found in process of fragmentation into mononucleated cells; 4) that the cytoplasmic relationship between the two cells is frequently very intimate; 5) that such intracellular normoblasts are occasionally in mitosis, an unexpected phenomenon in ingested degenerating cells; and the possibility 6) that the cells interpreted as phagocytic endothelia may indeed be cells differentiating normoblasts intracellularly while still attached to the blood vessel wall—all indicate that the structure in question is one representing actual intracellular differentiation of normoblasts within a giant cell. This matter will be further discussed below.

In the yolk-sac of the 25 mm. pig embryo the blood vessels are relatively much larger. No blood-islands occur. The blood cells are predominantly of the normoblast type; there are also some erythroblasts and a few haemoblasts. Giant cells are apparently lacking; and the endothelium of the blood channels is apparently no longer capable of haemoblast formation.

IV. DISCUSSION

a. Function of yolk-sac

1) *Digestive.* The yolk-sac entoderm is of course continuous with the epithelial lining of the gut through the yolk-stalk. Originally similarly undifferentiated, the yolk-sac entoderm already at the 5 mm. stage has far outstripped the gut entoderm in differentiation. Even at the 10 mm. stage the cells lining the gut are relatively little differentiated. The chief mark of functional activity on the part of the yolk-sac entodermal cells is the presence of a generous amount of basal filaments. Such are lacking in the gut entoderm of this stage. These filaments resemble very closely mitochondria; they may be long or short, straight or variously curved, delicate or coarse, apparently

homogeneous or segmented. While structurally very like mitochondria—on the basis of which characters I previously so interpreted them— I now feel compelled to give them a different interpretation, and for the following reasons: 1) Identical filaments appear in the cells of the hepatic cords (compare figs. 31 and 32) and those of the mesonephric tubules of these embryos. These cells are functionally active in a secretory way, strengthening the presumption that the filaments in the yolk-sac entoderm also have secretory significance. 2) If these filaments were really mitochondria, many other cells should show such elements, for it is well established that mitochondria are practically universally present in embryonal cells. But no other cells, besides those mentioned, contain similar filaments in these embryos. It is quite unreasonable to suppose that the technic should have preserved mitochondria only in selected types of cells. The filaments in question most probably have nothing directly to do with mitochondria. 3) The filaments are apparently identical with the ergastoplasmic filaments described by Bensley (1) for the parenchymal cells of the pancreas of the adult guinea pig, readily distinguishable from mitochondria demonstrable by appropriate technics. Similar filaments have been described for other secretory cells, as for example, salivary glands and kidney.

On the basis of the above considerations the conclusion seems unavoidable that these filaments in the yolk-sac entoderm are of secretory significance. The manner in which they function in the secretion process is uncertain, but there is some evidence that they segment distally into granules. These filaments, then, may be presecretion filaments. In the 25 mm. stages, filaments are relatively less, and granules relatively more, abundant than at the 10 mm. stage.

Similar structures have been described in the human yolk-sac of about this same stage [Jordan (10, 11, and 12); Branca (2)]. Branca indeed interpreted them as 'functional protoplasm.' I first designated them by the term 'mucinous masses,' since they reacted to the specific stains for mucus. In my first study (1907) I inclined to the belief that they were degeneration prod-

ucts. In the light of the data from pig embryos my subsequent ('10) interpretation as secretory structures appears to have been correct. The filaments have a basophilic staining reaction, hence stain well in specific mucous dyes. In later developmental (functional) stages they are limited to the basal ends of the cells, where they may become clumped into a deep staining irregularly oval mass. The 'mucinous masses' described for the yolk-sac of 9 and 13 mm. human embryos are essentially the same structure as the presecretion filaments of the 10 mm. pig embryo; and their functional rôle is most probably secretory.

What then may be the meaning of the yolk-sac entoderm in terms of function? The additional evidence from the yolk-sac of the pig, further supports my earlier conclusion ('07) that this cell structure is to be interpreted in terms of the ancestral history of higher mammals. In the ancestors with yolk laden eggs the entodermal cells undoubtedly had the function primarily of elaborating a digestive fluid for the liquefaction and assimilation of the yolk. In yolkless umbilical vesicles, the entoderm apparently still develops and differentiates in accord with an 'ancestral memory,' though it can perform no true digestive function. The umbilical vesicle of the pig, as of man, is in large part—that is, as concerns digestive significance—a vestigial structure. But it has taken on a secondary function, now apparently become of great importance, as an early, perhaps original, center of haemopoiesis.

The above discussion would seem to dispose of Paladino's (20) suggestion that the yolk-sac entoderm of higher mammals has a hepatic function. The form and structure of the two classes of cells are indeed very closely similar (figs. 31 and 32), but this need not necessarily imply identity of function. The similarity is due more probably to the fact of common origin from the primitive gut, and the further fact that both are functionally active, and in a secretory manner. Nor need the presence of glycogen in both types of cells be interpreted in terms of functional identity, since many types of cells of embryos contain glycogen [Gage (8)].

Neither brush borders nor terminal bars occur on these cells. Such structures have been described for the entodermal cells of the human yolk-sac by Branca (2). However in my own specimens of the human yolk-sac (10, 11, and 12), I could never convince myself of the presence of these structures. Lewis (15) likewise was unable to find them in human yolk-sacs of similar ages.

The entodermal cells in the yolk-sac of the 10 mm. pig embryo are undergoing extensive mitotic proliferation. This fact, viewed in conjunction with the good cytologic preservation of the cells as indicated primarily by the abundance and character of the presecretion filaments, should remove all doubt as to the normal and healthy condition of these specimens.

Not a single entodermal cell can be found in process of amitotic division. Nor are any of these cells binucleated. This is significant in view of the fact that all types of cells in the mesenchyma and its derivatives show abundant examples which admit of interpretation in terms of direct division.

2) *Haemopoietic*. The first question under this caption concerns the origin of the angioblast. The term 'angioblast' is employed here to designate the original anlage of the vascular tissue in the yolk-sac. It is obvious that no sharp line can be drawn between original and secondary angioblast. Suffice it to note that angioblast is still in process of formation in the yolk-sac of the 10 mm. embryo. No information accrues from this study touching the question of the origin of the first mass of vascular anlagen. Once formed, angioblast can of course spread by process of growth. However, it is also still being added to by previously discrete moieties. If these additions can be shown to be made from the mesenchyma, it would seem to afford a strong presumption against the derivation of the original angioblast from entoderm [Minot (19)]. Such anlagen do arise by differentiation within the mesenchyma in the shape of discrete blood-islands, as described above. I conclude for the mesenchymal origin of the angioblast on the basis, then, mainly of these two observations: 1) the common origin of endothelium and haemoblasts, as described above, from mesenchyma:

2) the sharp demarcation between mesenchyma and entoderm in the embryos here considered. Where blood vessel and entoderm abut, the basement membrane of the entoderm and the endothelial cells of the vessel are never in direct continuity (fig. 2).

The close detailed structural similarity between the mesothelial cells and the endothelial cells, and between the nuclei of both and those of the mesenchyma, was noted above (figs. 13 to 20). The criteria which Clark (4) applied in the chick embryo for the differentiation between endothelial nuclei and mesenchymal nuclei are inapplicable to the yolk-sac mesenchyma of pig embryos of the 5 to 15 mm. stages of development. Number of nucleoli, character of nucleolar contour, and depth of tingibility of nucleoli are not features by which mesenchyma nuclei can be differentiated from endothelial nuclei. These are marks which characterize different cells (probably representing different functional phases) of mesenchyma, mesothelium and endothelium alike.

The morphologic evidence seems to force the conclusion that endothelium and mesothelium are both very similar differentiation products of mesenchyma, the factor chiefly operative in the differentiation being the mechanical factor of pressure, as maintained by Huntington (9), Schulte (21) and others. The pressure exerted upon the mesothelium operates from the relatively more rapidly growing entoderm; that upon the endothelium from the confined blood cells and plasma. The further fact that haemoblasts arise from both mesenchyma and endothelium supports the conclusion of their essential identity.

If the above is correct then one would expect that the mesothelium also could produce haemoblasts. My material yields no data in support of this view. Indeed very careful study of the mesothelium both of the yolk-sac and the chorion, with this point in view, gave only negative evidence. The mesothelial cells proliferate both mitotically and apparently amitotically but nothing appears closely similar to the phenomena described by Bremer (3) for the chorion of the young human embryo, where the mesothelium is said to invaginate the underlying

mesenchyma of the body stalk in the form of cords and tubules (angiocysts) the cells of which differentiate into haemoblasts and endothelium. Bremer's observations, however, are a further very strong support to the claim that angioblast is of mesenchymal origin, and that mesenchyma, mesothelium and endothelium are originally identical structures.

The monophyletic theory of blood cell origin considers the haemoblast the common parent of both erythrocytes and leucocytes. Its correspondence with fact, at least in essential outlines, is now widely accepted. The point which has stimulated most discussion concerns the origin of isolated haemoblasts within the mesenchyma. Are such differentiation products of the mesenchyma, or are they migrants from the blood vessels? The latter view was held by Minot (19); Maximow (16 and 17) and others champion the opposing view. In the case of the yolk-sac of the pig, the evidence seems definite in favor of the *in situ* differentiation of haemoblasts from the mesenchyma. The observations both from blood-islands and single cells have been given above. Haemoblasts of course are capable of amoeboid activity, and undoubtedly do leave the blood vessels under certain conditions, and invade the surrounding mesenchyma. But that the cell (*h*) illustrated in figure 30 cannot be interpreted as such is clear from: 1) the connection of its nucleus, through a delicate chromatic bridge, with the nucleus of the mesenchyma; and 2) its perfectly healthy condition, both from the viewpoint of its nucleus and its cytoplasm. Nor can there remain any doubt that it is actually a haemoblast when its cytoplasm and nucleus, in contrast to the cytoplasm and nucleus of the mesenchyma, is compared with an intravascular haemoblast.

The evidence given above for the extensive origin of haemoblasts from the endothelium seems conclusive for the 10 mm. pig embryo. Neither at earlier nor later stages is this process so evident.

The haemogenic activity of the endothelium in the yolk-sac of the pig is of cardinal significance especially in view of Stockard's (23) findings in the case of the *Fundulus* embryo, where the problem was approached by the experimental method. This

consisted in the stoppage of the embryonic circulation by means of anaesthetics. Stockard's observations led him to conclude that in the *Fundulus* embryos investigated (up to 20 days) the endothelium plays no haemogenic rôle. In the pig embryo, on the contrary, the data leaves no escape from the opposite conclusion, a conclusion arrived at also by many investigators of various embryo forms. [e.g., certain chelonians, Jordan and Flippin (13)]. This conclusion is supported by the further important fact that the endothelium of the sinusoids of the liver and of the glomerular capillaries of the mesonephroi also produce haemoblasts.

The sole alternative interpretation that has any appearance of plausibility respecting the haemoblasts of the yolk-sac vessels here described as separating from the endothelium, is that they have become pressed against the wall and thus modified in shape and caused to adhere intimately to the endothelium, so as to stimulate endothelial continuity and derivation. This suggestion is rendered inapplicable by 1) the possibility of tracing a complete series of transition stages between a true endothelial cell, through intermediate haemoblast stages, to a free haemoblast; 2) the possibility of tracing a similar series through to multinucleated giant cells; 3) the fact that such haemoblasts in apparent continuity with the endothelium are quite as abundant in essentially empty vessels as in vessels crowded with blood cells, where alone an adequate factor of pressure would seem to prevail, and 4) that haemoblasts, though apparently naked cells, do not in general exhibit adhesive properties except among themselves.

b) Giant cells. The derivation and the morphologic and cytologic variations of the giant cells are clear, as described above. These cells are simply modified haemoblasts, capable of undergoing a similar differentiation into giant erythroblasts, and apparently ultimately differentiating normoblasts intracellularly. This last point may be thought perhaps to remain somewhat doubtful, and even if the interpretation is accepted, the significance and economy of this process—for it is clearly not essential, since it is not the exclusive method for yolk-sac haemopoiesis—still remains obscure.

That the giant cells have no genetic relationship to the entoderm, as urged by Graf. v. Spee (22), is certain. That the method of nuclear multiplication is largely a matter of budding and fission is also demonstrable (fig. 35). It may be stated also that these cells are much more abundant at about the 10 mm. than at earlier and later stages; and that while all the other types of erythrocytes are found in the embryonic circulatory system, giant cells are practically limited to the yolk-sac vessels. A few smaller varieties appear in the liver and the mesonephroi, and occasionally one appears in a capillary in the mesenchyma next the brain. Haemoblasts also are only sparingly found outside of the yolk-sac, liver, and the glomerular sinusoids of the mesonephroi. The normoblasts are in the vast majority in the intraembryonic circulatory system.

On the basis of their occasional normoblast content the giant cells might be interpreted 1) as erythrophages or 2) as multiple erythroblasts. The latter interpretation was urged by Graf v. Spee (22). The former interpretation is supported by the fact that endothelial cells—which are potential haemoblasts—may apparently function as phagocytes for erythroblasts. The latter more plausible conclusion rests upon my observations that in a giant cell with two normoblasts (fig. 26) no additional nucleus is present; and the further fact that frequently the cell membrane of the normoblast is not separated from the cytoplasm of the giant cell by any space, but the two structures appear continuous (fig. 26). Moreover in certain multinuclear giant cells the several nuclei and their enveloping cytoplasmic areas are at different stages of development. In figure 25 two of the nuclei are typical haemoblast nuclei, two are typical erythroblast nuclei. The upper right hand nucleus (*x*) is differentiated more than the other, and the enveloping cytoplasm is beginning to take on normoblast characteristics. Nevertheless this interpretation must perhaps still be regarded as more or less tentative. But the fact that mega- and polykaryocytes are present in all haemopoietic foci, embryonic, foetal and adult, strongly supports the conclusion that they are closely associated with the haemopoietic process. As such, however, their function does not seem

to be an essential one; they may represent simply atypical or possibly ancestral phenomena. Erythrocytes commonly develop from mononuclear haemoblasts; binucleated haemoblasts apparently sometimes divide to form two haemoblasts (fig. 3. e); multinucleated haemoblasts (polykaryocytes) do not break up into mononuclear haemoblasts, but may produce erythrocytes (normoblasts) intracellularly.

V. SUMMARY

1. In pig embryos of about 10 mm. length, the yolk-sac attains its highest stage of progressive histologic differentiation. This statement pertains both to the entoderm and to the angioblast.

2. The entodermal cells are characterized chiefly by abundant presecretion filaments, in which feature they agree with the cells of the liver and mesonephroi.

3. Angioblast arises from the mesenchyma.

4. The mesothelium of the yolk-sac of pig embryos between 5 and 12 mm. does not produce haemoblasts. Nor is there any satisfactory evidence that the mesothelium of the body stalk and chorion function to this end.

5. The mesenchyma may differentiate directly into endothelium or into haemoblasts.

6. Haemoblasts arise extensively at the 10 mm. stage from the endothelium of the yolk-sac blood vessels. The endothelia of the hepatic sinusoids and mesonephric glomeruli of this stage also show extensive haemopoietic capacity.

7. Giant cells, both mono- and polynuclear, are abundantly present in the yolk-sac only at about the 10 mm. stage of development. They may arise from endothelium or directly from haemoblasts. They are giant haemoblasts, and apparently function as multiple erythroblasts in which normoblasts differentiate intracellularly.

8. The several stages in haemopoiesis, represented successively by haemoblasts, erythroblasts and normoblasts, with transition stages, are abundantly present in the yolk-sac of embryos from 5 to 15 mm.

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PLATE 1

EXPLANATION OF FIGURES

(Unless otherwise specified the illustrations are from a single specimen of the 10 mm. stage, the magnification 1000, the fixation with Zenker's fluid, and the stain employed the haematoxylin-eosin combination).

1 A group of blood cells from one of the larger yolk-sac vessels of a 6 mm. pig embryo (Helly's fixation; Giemsa stain; magnification, 1500 diameters). a) various types (differentiation stages) of haemoblasts; the sparse naked cytoplasm has a vague irregular granular character and stains intensely blue; the large vesicular nucleus stains a very light blue and contains a delicate, finely granular reticulum and one or several spheroidal or irregular nucleoli staining like the chromatic granules, a bluish orange. b) Young erythroblasts ('megalo-blasts'); the nucleus is relatively smaller and the cytoplasm more voluminous than in the smaller younger haemoblasts; the cytoplasm stains a light blue (brownish gray or bluish pink) and contains fine, uniform, spherical granules (probably haemoglobin); a cell wall is distinct; the still vesicular nucleus contains a coarsely granular reticulum which stains blue; some of these nuclei still contain a nucleolus. c) Older erythroblasts; the nucleus has become still smaller and more chromatic; the homogeneous cytoplasm is relatively more voluminous and now stains pink. d) Normoblast; the nucleus is small, granular and chromatic; the cytoplasm stains brilliant red (in Zenker fixed tissue the cytoplasm consists merely of a coarse irregular unstainable reticulum enclosed by a robust cell membrane.)

2 Narrow portion of wall of yolk-sac including all of its layers. *E*, entoderm; the cells contain many presecretion filaments. Between the entoderm and peripheral mesenchyma is a large blood vessel containing a few blood cells at various stages in the metamorphosis into a normoblast (*e*); a) endothelial cell; b) haemoblast; c) binucleated haemoblast with long pseudopod; d) binucleated erythroblast. *M*, mesothelium; *bm.*, basement membrane; *end.*, endothelium.

3 A group of developing blood cells from a yolk-sac blood vessel. a and b) young haemoblasts; c) haemoblast with nucleus in process of amitotic division; d) binucleated haemoblast; e) binucleated haemoblast in process of cytoplasmic amitotic constriction, a fairly common form of cell; f) erythroblast (Maximow's 'megalo-blast'); g) normoblast.

4 Portion of wall of yolk-sac of 10 mm. pig embryo including mesothelium, endothelium and the intervening mesenchyma. a) endothelial cells from wall of a blood vessel; b) endothelial cell in early stage of separation from wall of blood vessel to become a haemoblast; c) later stage; d) extravascular haemoblast, separating from the endothelium.

5 Haemoblast at late stage in process of separation from the endothelium.

6 Haemoblast, of spindle shape, just about to separate from the endothelium.

7 Uninucleated giant cell; large haemoblast.

8 Trinucleated haemoblast (giant cell) in final stage of separation from the endothelium (*e*).

9 Trinucleated giant cell, immediately after separation from endothelium. Note the lateral basal projections, the points of final separation. One of the nuclei is apparently undergoing amitosis.

10 Binucleated haemoblast in which one of the nuclei and the surrounding cytoplasm have differentiated into a normoblast.

11 and 12 Trinucleated giant cells.

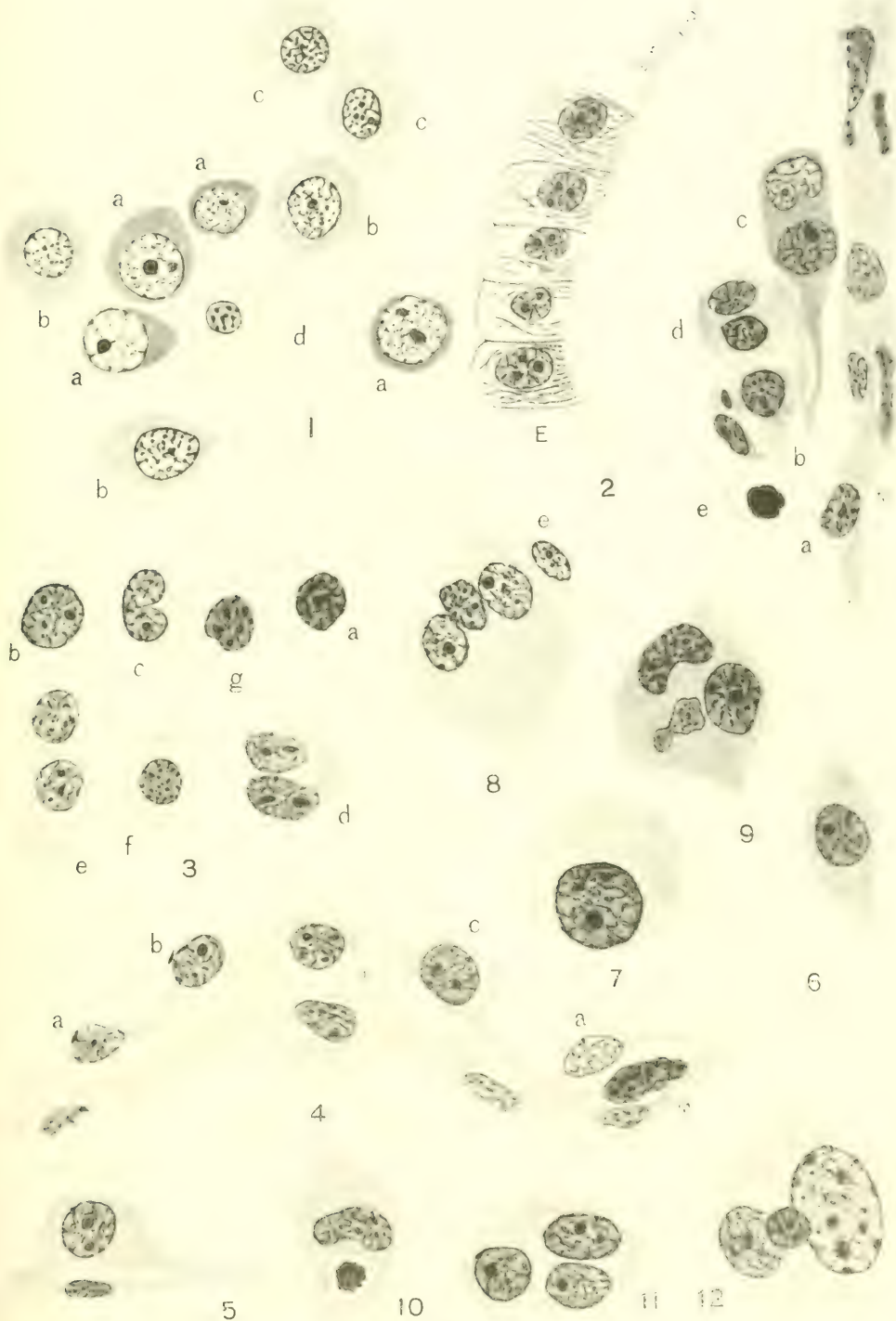


PLATE 2

EXPLANATION OF FIGURES

13 Haemoblast (*h*) in final stage of separation from endothelium; *e*, endothelial cell.

14, 15 and 16 Three successive stages in the transformation of an endothelial cell into a haemoblast. *E*, towards entoderm; *V*, towards blood vessel.

17 Nucleus of endothelial cell in phase of amitotic division. Many nuclei also can be seen in mitosis.

18 Nucleus from mesenchyma. Note the similarity between nuclei of endothelium, mesothelium and mesenchyma.

19 and 20 Two mesothelial cells. *s*, towards surface. Occasional cells can be seen in mitosis.

21 Haemoblast in mitosis. The spindle is apparently tripolar. Such irregular mitoses if sufficiently common would explain the multinuclear haemoblast with nuclei of various sizes. Haemoblasts apparently divide both mitotically and amitotically.

22 Haemoblast with nucleus apparently dividing amitotically.

23 Large binucleated giant cell; the cytoplasm is at an early phase of differentiation into the erythroblast type; the nuclei also are in early, but different, stages of differentiation.

24 Smaller binucleated giant cell (haemoblast); the nuclei are of the typical haemoblast type.

25 Giant cell from yolk-sac of 10 mm. pig embryo with four nuclei, which, with their enveloping cytoplasm, are at different stages of differentiation. Two of the nuclei have haemoblast characters, one erythroblast and one (*x*) early normoblast characters. The cytoplasm also around *x* has normoblast characteristics.

26 Binucleated haemoblast (giant cell) in late stage of process of direct intracellular differentiation into two normoblasts.

27 Haemoblast with one long and several shorter stubby pseudopods.

28 Endothelial phagocytic cell (perhaps a differentiating haemoblast) having ingested an erythroblast whose nucleus is undergoing karyorrhexis, the cytoplasm appearing normal.

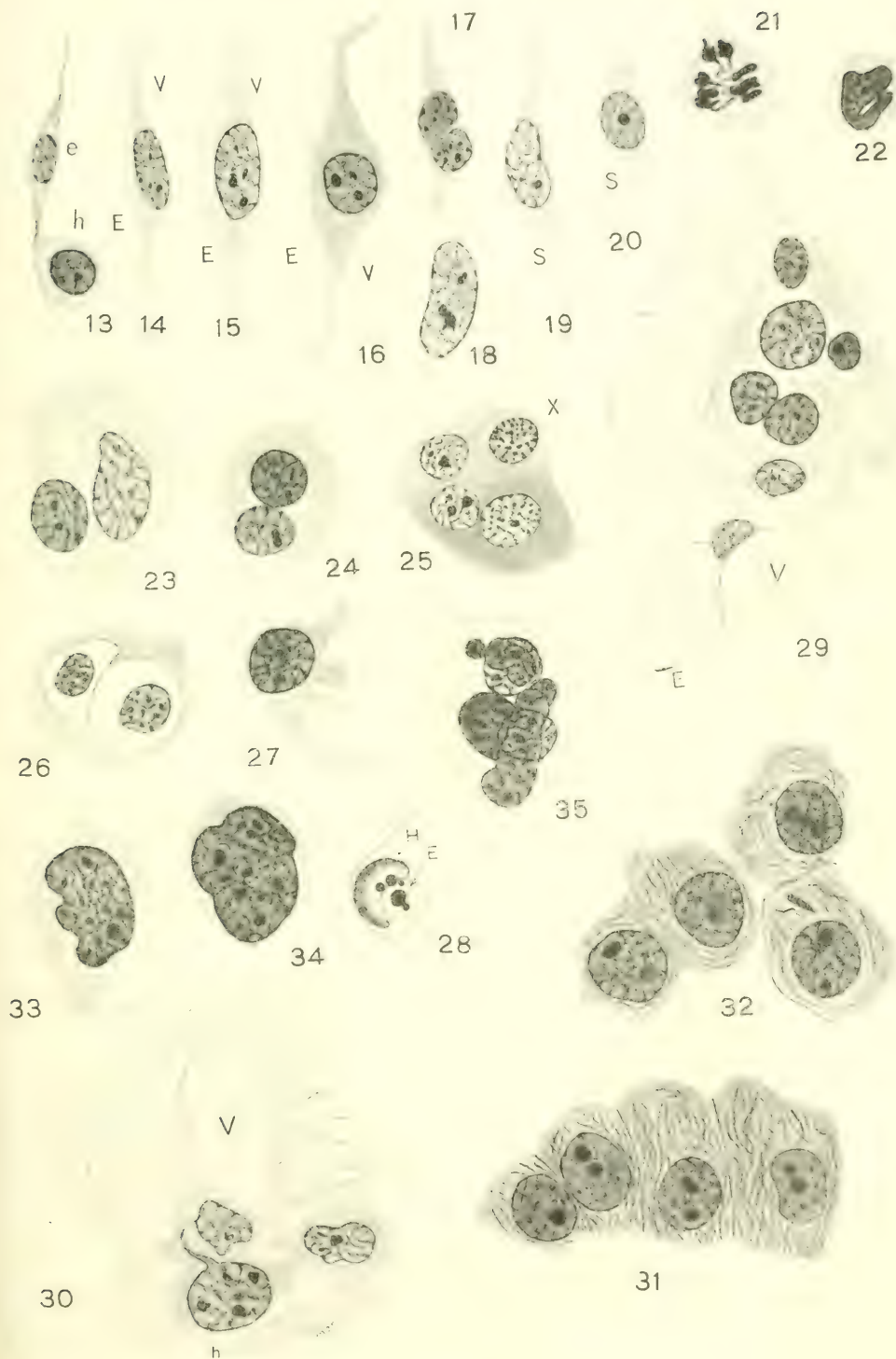
29 Portion of wall of yolk-sac of 10 mm. pig embryo showing a small blood island. The cells are all of the early haemoblast stage, and closely related peripherally to the surrounding mesenchyma, from which they have apparently differentiated. One haemoblast is binucleated. *E*, entoderm, schematically represented; *V*, blood vessel.

30 Portion of wall of yolk-sac of 10 mm. pig embryo showing the differentiation of a haemoblast (*h*) from the mesenchyma. The nucleus of the definitive haemoblast is still connected through a chromatic nuclear strand with the nucleus of its sister mesenchymal cell. *E*, entoderm, schematically represented; *V*, blood vessel; *mes.*, mesothelium.

31 Four adjacent entodermal cells to show especially the 'basal' or presecretion filaments.

32 A group of four adjacent liver cells from the same embryo, to show the close similarity in nuclear and cytoplasmic structure and form between the hepatic and yolk-sac embryonic epithelium. Many of the hepatic cells (not here represented) show mitotic figures; amitotic divisions apparently do not yet occur.

33, 34 and 35 Various types of giant haemoblasts. Figure 35 is typical of a large group of giant cells whose nuclei proliferate amitotically.



EFFECTS OF INANITION UPON THE STRUCTURE OF THE THYROID AND PARATHYROID GLANDS OF THE ALBINO RAT

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FOURTEEN FIGURES

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INTRODUCTION

The thyroid gland presents an interesting and difficult biological problem. Although the morphology of the thyroid has been extensively studied, there are still many doubtful and unsettled questions concerning its development and its normal adult structure. Even more uncertainty exists concerning its physiological significance and its pathological changes. Some light may be thrown upon the various phases of this problem by a study of the changes produced in the thyroid gland by inanition.

In previous papers (Jackson '15 a, '15 b) it was shown that during inanition the various organs of the albino rat suffer very unequally in loss of weight, the loss also varying according to

the length and character of the inanition. In acute inanition of adult rats, with loss of about one-third in body weight, the thyroid gland had apparently lost but little if any in absolute weight: in chronic inanition of adults, the average apparent loss of the thyroid was about 22 per cent; while in young rats held at maintenance (constant body weight) by underfeeding for several weeks the average loss was about 24 per cent. In order to determine what histological changes are correlated with these changes in gross weight, material was preserved for further study. On account of its intimate association with the thyroid, the parathyroid gland was also included in this investigation. The results are presented in the present paper, which is the third of a series of studies upon the effects of inanition. The work is being carried on with the assistance of a special grant from the research fund of the Graduate School of the University of Minnesota.

MATERIAL AND METHODS

The material used included the thyroid (and included parathyroid) glands of the albino rat (*Mus norvegicus albinus*) from previous studies (Jackson '15 a, '15 b), together with some collected since. In all, more than 50 normal glands were sectioned and studied, varying in age from newborn to adult (15 months). These were chiefly controls from the same litters as those used for experiments (including several controls used in experiments by E. R. Hoskins and C. A. Stewart). In addition, I am indebted to Professor Bensley and Mr. Burgett, of the University of Chicago, Professor Addison, of the University of Pennsylvania, and Professor Evans, of the University of California, for material kindly furnished in order to investigate possible local variations in normal thyroid structure of the rat.

Of the animals subjected to inanition, the thyroid and parathyroid were obtained from 14 of the younger rats held at maintenance for various periods (chiefly beginning at 3 weeks and ending at 10 weeks of age). Of the adult rats, 6 glands were

studied from those subjected to acute inanition, and 3 from those with chronic inanition.

The material was obtained at the autopsy held immediately after the animals were killed, and was fixed chiefly in Zenker's fluid, 12 to 24 hours. In a few cases formalin or Flemming's fluid was used, but the results were less satisfactory.

The glands were embedded in paraffin, and cut at 5 micra (occasionally 7 to 10 micra) in thickness. In the great majority of cases, the glands were cut and mounted in complete serial sections. This was found to be important, not only to make certain of including the parathyroids, but also because the structure frequently varies in different parts of the thyroid and parathyroid glands.

The sections were stained in most cases with haematoxylin and eosin; in a few cases with iron-haematoxylin, safranin, Mallory's anilin-blue connective tissue stain, etc.

All of the drawings were made with a Zeiss 2 mm. 1.30 N. A. apochromatic objective and compensating ocular No. 6, with the aid of a camera lucida. A wheel-micrometer eyepiece was used for the measurements. It was the original intention to measure systematically a large number of cells and nuclei in the glands of the controls and of the animals subjected to inanition. On account of the great irregularity in the size and shape of cells and nuclei, however, it was found that the results, though of limited value, could not be obtained with sufficient accuracy to justify any very extensive series of observations. Therefore the number of measurements was restricted to that judged sufficient to give merely an approximation of the apparent average and range observed.

THE THYROID GLAND

The normal structure of the thyroid gland in the albino rat at various ages will first be considered. Then the changes found in the various types of inanition will be described and their significance discussed.

a. Normal structure of the thyroid gland

The general form and topography of the thyroid gland in the albino rat is shown in cross section in figure 1. Each lateral lobe presents the typical relations—convex external surface covered by the infrahyoid musculature; concave internal surface in contact with the lower larynx and upper trachea; and narrower posterior surface (or border) in relation with the oesophagus

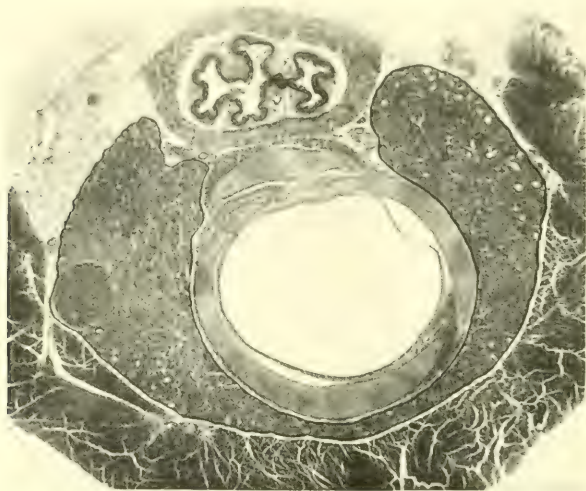


Fig. 1 From a photograph (retouched) of a cross section of the thyroid gland in situ at the level of the isthmus, showing relations to infrahyoid musculature, uppermost trachea, oesophagus, etc. One parathyroid is visible, on the left side of the figure. From albino rat No. S 9.47, age 22 days, gross body-weight 25.5 grams. ($\times 28$.)

medially the carotid artery, etc., laterally. The isthmus is frequently a very thin somewhat fibrous band, almost invisible when the fresh gland is exposed in situ. It invariably contains thyroid follicles (contrary to Sobotta '15), although these may become scattered and more or less atrophied in adult rats.

The minute structure of the normal thyroid gland at 3 weeks (the age when the experiments began with the younger rats) is shown with slight magnification in figure 1, and under high power in figure 2. No attempt will be made to describe in detail the

minute structure of the gland, but some of the essential features especially affected by inanition will be considered briefly.

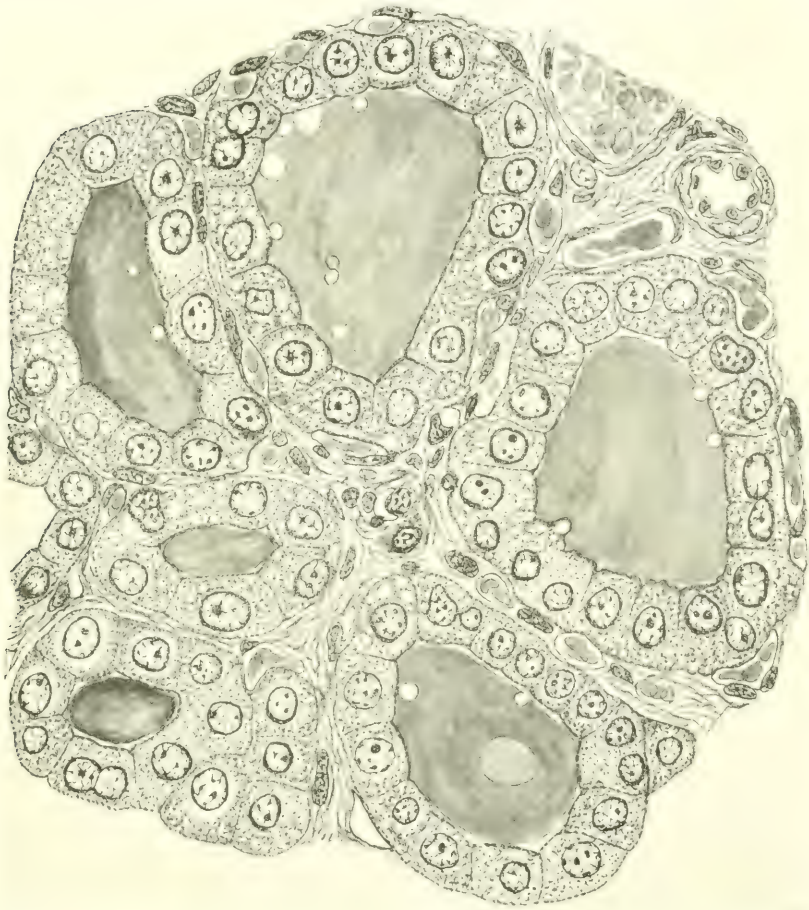


Fig. 2 A small portion of the thyroid gland shown in figure 1 (rat S 9.47, age 22 days) magnified to show the details of the normal histological structure. Several follicles containing colloid are shown. Follicular epithelium cuboidal; cytoplasm abundant and granular, with a few scattered vacuoles. Apparent origin of the colloid 'vacuoles' from the epithelial cells is shown in a few places. Four interfollicular epithelial cells are seen between the two lower follicles. Fibrous stroma scanty, with rich blood-vascular plexus. ($\times 750$.)

The follicles at this time are chiefly oval or rounded in outline and vary mostly from 20 to 70 micra in diameter. The larger follicles are rather infrequent and somewhat uniformly scattered, but are usually more frequent near the surface of the gland. Those shown in figure 2 are of average size.

The finer structure of the thyroid gland is shown in figure 2. The cells of the follicular epithelium are approximately cuboidal in form (in some cases low columnar; in others, especially in the larger peripheral follicles, somewhat flattened). In height, they range chiefly between 8 and 15 micra, the average being 10 to 12 micra. The inner and outer cell walls are sharply distinct; the intercellular boundaries are less distinct and sometimes absent.

The cytoplasm of the follicular cells (fig. 2) is filled with moderately fine granules, reddish violet in color (with Zenker fixation and haematoxylin-eosin stain). The granules are usually somewhat uniformly distributed. They are not densely packed, but are sometimes arranged so as to give an indefinite reticular form, apparently intermingled with small, clear vacuoles. Some of these vacuoles (though not all) may correspond to the minute fat droplets or granules described in the thyroid cells by Erdheim ('03) and Traina ('04). The cells present a fairly uniform appearance, and there is nothing to indicate any division into the 'chief' and 'colloid' cell types of Langendorff.

The nuclei of the follicular cells are spherical or slightly ovoidal (ellipsoidal) in form, the diameters varying from 4 to 7 micra, usually 5 or 6 micra. The nuclear membrane is distinct and stains deeply. There are usually one or two larger nucleoli (karyosomes) and several smaller granules; and a fine, paler nuclear network, often indistinct, with a very pale bluish, homogeneous nuclear background, corresponding to the nuclear sap (karyolymph). The nuclei shown in figure 2 are typical, though in the larger peripheral follicles with slightly flattened cells the nuclei may also be somewhat more flattened and slightly hyperchromatic. Cells in mitosis are relatively frequent, 5 having been noted in one entire cross-section of one lobe, and 6 or 8 in another.

The colloid (fig. 2) appears typical in form though somewhat variable in staining reactions. In some cases it fills the follicular cavity completely, in other cases it is retracted somewhat with either smooth or serrated margin. This retraction is probably an artefact in most cases, due to shrinkage produced by the reagents used. But I cannot agree with those investigators who explain the vacuoles (some of which are shown in figure 2) in a similar manner. These vacuoles are usually small (4 micra or less) and spherical in form, and are most frequent near the surface of the colloid. Occasionally they are found in intimate relation with the adjacent cells, from which they are apparently extruded, as shown in the follicle on the right in figure 2. They are probably connected in some way with the process of colloid formation, as described by Anderson ('94) and Müller ('96). Desquamated epithelial cells, which sometimes dissolve leaving clear vacuoles in the colloid in older rats, are extremely rare at this stage.

A variable amount of interfollicular epithelium appears, which cannot be distinguished from tangential sections of follicles, except in serial sections. In structure, these interstitial epithelial cells are similar to those of the follicles. A few appear in the lower part of figure 2.

The interfollicular connective tissue forms a delicate fibrous stroma (fig. 2), relatively small in amount, but containing a rich capillary plexus of blood-vessels. The nuclei visible are mostly of capillary endothelium. They are elongated or flattened in form, and stain somewhat deeply.

At 10 weeks (the age when the inanition experiments ended in most of the younger rats) the thyroid gland has normally undergone but slight changes, the structure being essentially the same as that just described at three weeks. Therefore no detailed figures are considered necessary. A photograph with low magnification is shown in figure 3, representing a cross section of one lateral lobe. The follicles have increased somewhat in size, the maximum diameter now reaching about 100 micra. The larger follicles are more frequent, and are sometimes rather uniformly distributed, as shown in figure 3, though very frequently



Fig. 3 From a photograph of one lobe of the thyroid gland from normal albino rat No. S 5.3, age 74 days, gross body-weight 172 grams. Parathyroid included. Compare with figures 4, 5 and 6, representing thyroids in rats of the same age, but held at maintenance from age of 3 weeks. ($\times 28$.)

Fig. 4 From a photograph of one lobe of the thyroid gland from albino rat No. S 11.63, age 72 days, gross body-weight 23.8 grams (held at maintenance from age of 3 weeks). Follicles much larger at periphery. Some extra-capsular tissue is included. Parathyroid relatively large. ($\times 28$.)

there is a distinct tendency to larger follicles in the superficial layers.

In finer structure, the thyroid at 10 weeks is very similar to that shown at 3 weeks (fig. 2). The height of the follicular cells varies considerably, however. While the maximum is about the same as at 3 weeks, the average (about 8 to 10 micra) is somewhat lower. In other words, the cells are usually more flattened, especially toward the periphery of the gland. In the larger surface follicles, the cells are usually distinctly flattened, 6 micra or less in height. The nuclei in these cells are also correspondingly flattened, their diameters averaging 4×6 micra. The nuclei in general are similar to those at 3 weeks in size and structure, usually nearly spherical in form, and averaging about 6 micra in diameter. Mitosis is very much less frequent in the cells of the thyroid at 10 weeks than was found in the gland at 3 weeks.

While the typical normal structure of the thyroid cells at 10 weeks is like that at 3 weeks (fig. 2), cells of abnormal appearance are also found. These atypical forms vary from slight modifications up to marked cellular degenerations, and should be carefully noted in order to avoid confusion with the changes during inanition to be described later.

The flattening of the epithelium in the larger peripheral follicles is almost constant, though variable in extent. The flattened nuclei are hyperchromatic in type, and often present a more or less deeply-staining, homogeneous background, which may obscure or obliterate the nuclear network and granules. The cytoplasm is reduced in amount, more deeply-staining, and often somewhat homogeneous in appearance. This type of cell occurs so constantly in the peripheral follicles (and occasionally elsewhere) that it can hardly be considered abnormal. I inter-

Fig. 5 From a photograph of one lobe of the thyroid gland from albino rat No. S 5.10, age 67 days, gross body-weight 22.7 grams (held at maintenance from age of 3 weeks). Gland small, with relative increase of stroma. Parathyroid included. ($\times 28$.)

Fig. 6 From a photograph of both lobes and a portion of the isthmus of the thyroid gland from albino rat No. S 11.64, age 73 days, gross body-weight 24.2 grams (held at maintenance from age of 3 weeks). Follicles larger at periphery but irregular, many degenerated. Parathyroids relatively large. ($\times 28$.)

pret it as an atrophic type, due perhaps largely to the pressure on the gland from adjacent organs. Although these follicles are filled with dense, deeply-staining colloid, it is unlikely that the flattening is due entirely to consequent endofollicular pressure, as follicles are sometimes seen in which the epithelium on the external surface is much more flattened than that on the inner aspect of the follicle. The so-called 'colloid' cells of Langendorff (frequently described by various authors) probably belong to this atrophic type, and have no specific functional significance.

In addition to these peripheral flattened atrophic cells, mention must be made of more advanced types of degeneration, although the latter appear much less frequent in the young rat at 10 weeks than in older animals. These degenerative cells may occur in any part of the gland, either singly or involving an entire follicle (occasionally a regional group of follicles). The degenerating cells may remain in the follicular wall or may be desquamated into the follicular cavity. In rare cases the desquamated epithelium may replace the colloid with an irregular mass of cells in various stages of degeneration.

In the degenerating cells, the cytoplasm loses its typical light granular structure and becomes vacuolated and reticular in appearance, later disintegrating into irregular, usually deeply-staining (eosinophile) masses. The nucleus may be hypochromatic (karyolytic) in type, but more frequently presents various grades of pycnosis (rarely karyorrhexis), especially in the desquamated cells.

As to the frequency with which these degenerative types of cell occur in the thyroid of (apparently) normal rats at 10 weeks, it may be stated that of 9 glands carefully examined in serial sections, one showed rather extensive degeneration, one a well marked area (much less extensive), and four showed traces or small areas in early stages of degeneration. Thus in a majority of the glands, at least slight traces of degeneration could be found, even in normal, apparently perfectly healthy animals.

In older rats (from 3 to 15 months of age), the normal structure of the thyroid gland is essentially similar to that described for the younger rats. The follicles average slightly larger, the

maximum sometimes reaching 150 micra, the largest follicles being frequently found at the periphery of the gland. While cubical cells still occur to a variable extent, the average cell is somewhat more flattened than at 10 weeks, especially in the larger peripheral follicles. The colloid is variable in appearance. Occasionally the follicular content may be clear and unstainable. The stroma remains as previously described.

The most striking difference in the thyroid of the older rats is found in increasing prevalence of the degenerative process already described as appearing at 10 weeks. In the older control rats it was found not only more frequently, but more pronounced in character. In extreme cases, the follicles in some regions (see fig. 10) are entirely obliterated, being replaced by masses of epithelial cells with irregularly disintegrated cytoplasm and nuclei in various stages of pycnosis or karyorrhexis. Among 20 glands from apparently normal older rats, only 3 thyroids appeared entirely normal; 6 showed slight degeneration, 5 were moderately involved, while in 6 the degeneration was extensive, involving the larger portion of the gland. The significance of this appearance of degeneration in normal animals will be discussed later.

b. Structure of thyroid gland in young rats held at maintenance

The appearance of the thyroid gland in young rats held at constant body-weight from 3 to 10 weeks of age is shown under low magnification in figures 4, 5 and 6, and more highly magnified in figures 7, 8 and 9. The follicles in general appear in average size smaller than the normal at 10 weeks, although it is somewhat difficult to make exact comparisons, on account of the irregularity in their size. The maximum diameter found in 9 normal glands was about 100 micra, whereas in 14 maintenance rats it was 85-90 micra. The average in both cases was of course much lower, as follicles are found of all sizes down to those with only a minute cavity. Although in the maintenance rats at 10 weeks a few follicles are larger than found in the normal rat at the age of 3 weeks (where the maximum was 70 micra), it is doubtful whether the average follicle is any larger. It is probably slightly smaller.

In size, the epithelial cells of the thyroid follicles in the young rats held at maintenance are variable but distinctly smaller than normal. Even the largest cells found rarely reach the average

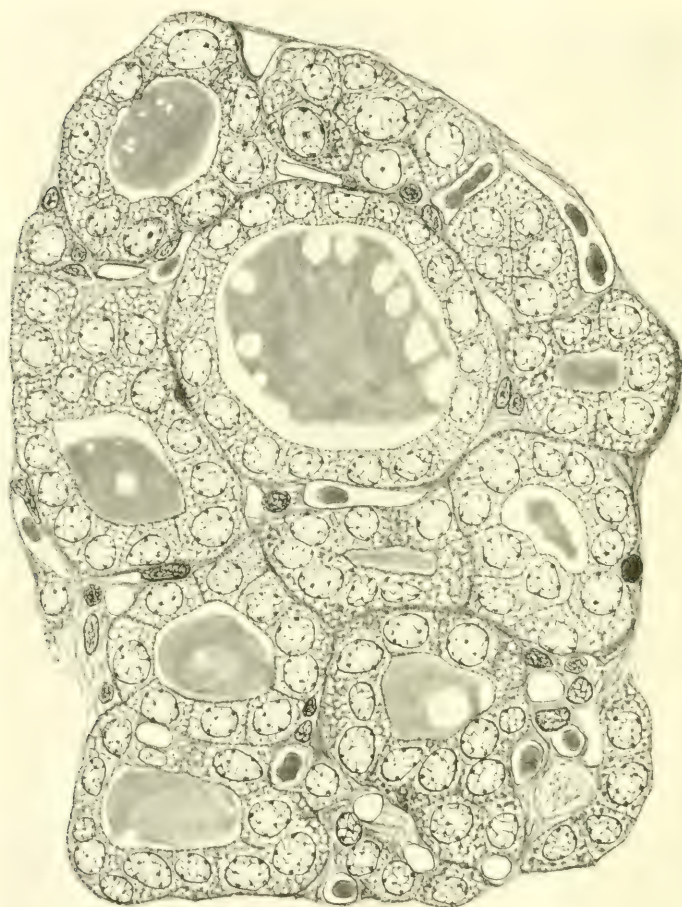


Fig. 7 A portion of the same thyroid gland shown in figure 4 (rat No. 11.63, maintenance from 21 to 72 days of age), magnified to show details of histological structure. The area represented shows the hypochromatic (incipient karyolytic) type of nuclear structure, which is relatively infrequent. Cells reduced in average height (cf. fig. 2). Cytoplasm reduced in amount and vacuolated in structure. Stroma here normal. ($\times 750$.)

normal height (8-10 micra) at corresponding age. The average height in the maintenance rats is about 6 to 7 micra. Since the

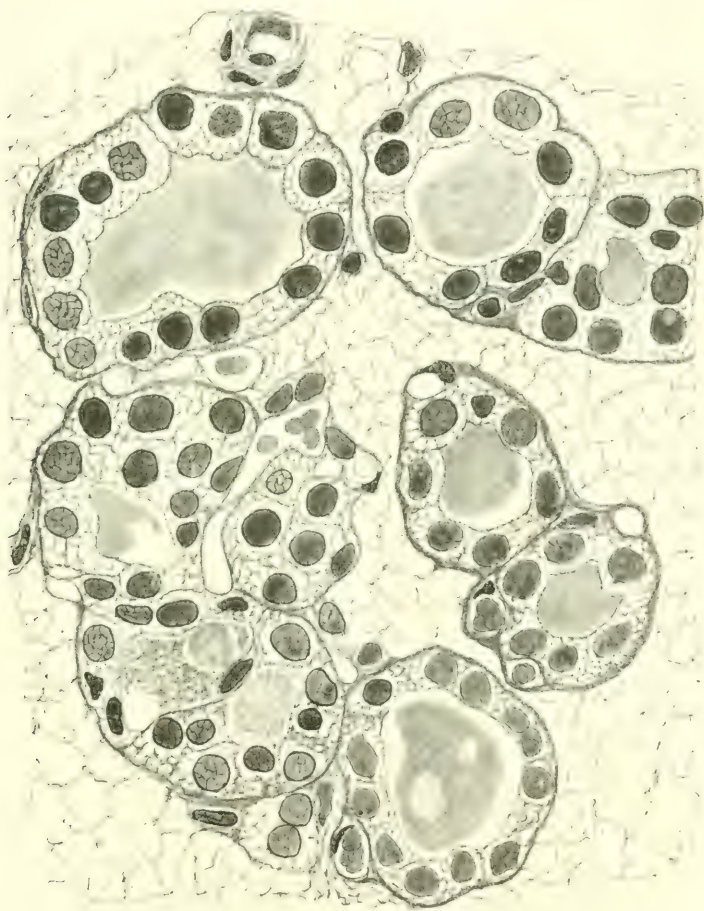


Fig. 8 A portion of the same thyroid gland shown in figure 5 (rat No. S 5.10, maintenance from 21 to 67 days of age), magnified to show details of histological structure. This represents the hyperchromatic (various stages of karyopycnosis) type of structure, which is typical. Follicular cells usually greatly reduced in size. Cytoplasm much reduced in amount and markedly vacuolated ('hydropic degeneration'). Colloid variable in appearance. Interfollicular stroma relatively much increased in volume, due to infiltration of clear ground substance. ($\times 750$.)

whole follicle is but slightly below the normal size, it follows that the cells are more reduced in height than in width. In some glands, the follicular epithelium is very strikingly flattened throughout the entire gland, in no place exceeding 4 or 5 micra in height. Such a condition is never found in the normal gland.



Fig. 9 A portion of the same thyroid gland shown in figure 6 (rat No. 11.64, maintenance from 21 to 73 days of age), magnified to show details of histological structure. This area represents advanced stages of follicular degeneration. Cells in various stages of degeneration and disintegration. Tendency to desquamation with destruction of colloid and obliteration of follicular cavity. Some nuclei appear karyolytic, although karyopycnosis predominates, and karyorrhexis frequently appears. ($\times 750$.)

In the larger peripheral follicles, in which the epithelium is normally flattened, the change is usually not so apparent in the maintenance rats. Even here, however, there is a further flattening of the epithelial cells, which frequently present an endothelial-like appearance, with a height of only 2 or 3 micra.

The cytoplasm of the thyroid epithelium in the maintenance rats has undergone marked changes (figs. 7, 8, 9). In comparison with the normal (fig. 2), it appears that the amount has been greatly reduced, the decrease in the size of the cell being due very largely to loss of cytoplasm.

The cytoplasm is also greatly changed in structure, having apparently undergone a 'hydropic degeneration.' The normal granular appearance of the cells (fig. 2) has been replaced very largely by a distinct reticular appearance (figs. 7 and 8). This appears to be due to a coarse vacuolization of the cytoplasm, a condition rarely found in the normal gland. The vacuolization is especially marked in the few larger cells which have retained more cytoplasm than usual. Occasionally the vacuoles coalesce to form perfectly clear perinuclear areas (see upper part of fig. 8). These clear areas and vacuoles probably represent a watery fluid replacing the normal granular substance, which has been removed and consumed as a result of the inanition.

Where the cytoplasm is very greatly reduced in amount (as in the larger peripheral follicles), the vacuoles may be finer or absent, and the cytoplasm here presents a denser, more deeply-staining (eosinophile), often homogeneous appearance ('colloid' type). In the case of cells in advanced stages of degeneration (which appear much more frequent and more pronounced than in the normal at this age), the cytoplasm is more or less disintegrated and extremely varied in appearance (fig. 9). It usually presents an irregular, deeply-staining (eosinophile), coarsely granular mass, which, especially in the desquamated cells, becomes fragmented and is gradually absorbed, frequently leaving behind the naked, pyknotic nuclei (fig. 9). The cell walls are often distinct in the less modified epithelium, but usually indistinct or lost in the cells markedly degenerated.

The nuclei, generally speaking, appear somewhat more resistant to inanition than does the cytoplasm. In form and size, there appears to be comparatively little change in many of the thyroid nuclei in rats held at maintenance from 3 to 10 weeks of age. The average diameter found in the nucleus of the more nearly cubical cells is between 5 and 6 micra, while the normal at corresponding age is only about 6 micra. It is therefore evident that the nuclear decrease in volume is relatively small. (A decrease of even 10 per cent in the nuclear diameter would correspond to a loss of about 27 per cent in volume, however.) In the more flattened cells (which, as we have seen, usually include the majority), the nucleus is also usually correspondingly flattened, with some loss in volume. Even in the flattened cells, however, the nuclear loss is apparently relatively much less than the cytoplasmic loss in volume.

Although the loss in volume of the nucleus in the thyroid of maintenance rats is relatively small, the structure is usually distinctly modified. Nuclei of the typical normal appearance (as in fig. 2) are rare. The modifications found are varied, but may be classified in two groups—hypochromatic and hyperchromatic.

The hypochromatic type is comparatively rare. In 14 cases of maintenance rats, it appeared extensively but once (shown in fig. 7), and in 3 other cases more or less frequently in scattered cells. The hypochromatic nuclei (fig. 7) usually appear normal in size, or even slightly swollen. They are very light and vesicular in appearance. The nuclear membrane is thin, but sharp and clear; the nuclear network and granules faint and indistinct. The interior of the nucleus is filled with a clear nuclear sap (karyolymph), which is nearly colorless, or with a very pale blue homogeneous tint. In exceptional cases these hypochromatic nuclei apparently undergo complete karyolysis, and disappear without preceding change in form or size.

The hyperchromatic form of nucleus on the other hand, is typical. In the 14 cases of maintenance rats, it appeared more or less extensively in thyroid of all (even in the one case in which the hypochromatic type predominated). Apparently the earli-

est stage (or at any rate that departing least from the normal type) consists in a mere thickening of the nuclear membrane ("nuclear wall hyperchromatosis") or an increase in the number and size of the nuclear chromatic granules, so that as a whole the nucleus appears darker and more deeply-staining than usual. This form appears normally in the more flattened cells of the larger peripheral follicles, and in the maintenance rats occurs more or less frequently throughout the gland, associated with the shrinkage and flattening of the cells (especially where the nucleus is also flattened).

Associated with or following the nuclear hyperchromatosis just described (in some cases perhaps independently thereof) is found a deepening coloration of the homogeneous nuclear background, which gives the appearance shown in figure 8. This appearance of incipient pycnosis is very characteristic and represents the predominant type of nuclear change observed. Apparently the process involves a progressive dissolution of the basic chromatin masses, which are probably dissolved in the nuclear sap. Various stages occur. The earliest show merely a slight darkening of the homogeneous background, which gradually increases until the nuclear net and karyosomes are obscured and finally disappear (figs. 8 and 9). In size, the nucleus at first shows no marked change (beyond perhaps a slight preliminary shrinkage), but as the process continues the nucleus usually decreases greatly in size. In the typical rounded, homogeneous, dense pycnotic nuclei, the diameter rarely exceeds 4 micra, which represents a tremendous shrinkage. When this stage is reached, the nuclei may apparently persist for a long time without further change, even though the cytoplasm has been reduced to a vestige or entirely removed (as, for example, in the mass of nuclei on the right in figure 9). In many cases of advanced cellular degeneration, however, the pycnotic nuclei become irregular in form and may later undergo karyorrhexis, breaking up into fragments which eventually through karyolysis are dissolved and disappear. Some of these extreme stages of karyorrhexis are shown in figure 9.

In no case was cell-division, either mitotic or amitotic, observed in the thyroid gland of the young rats held at maintenance for several weeks.

The colloid in the thyroid of the maintenance rats is usually nearly normal in appearance (figs. 7, 8), excepting those follicles with advanced degeneration of the epithelium. In these (fig. 9), especially where the epithelial cells are desquamated, the colloid becomes more or less granular and fragmented, finally appearing as irregular masses among the cellular debris or disappearing altogether. The follicles in advanced stages of degeneration are often very irregular in form.

The interfollicular (interstitial) epithelial cells (shown in figures 7 and 8), appear to undergo changes very similar to those in the epithelium of adjacent follicles.

The interstitial connective tissue or stroma of the thyroid in maintenance rats may be nearly normal in amount and structure (as in figure 7), but is frequently increased. This increase is apparently due to an infiltration of clear ground-substance, which in extreme cases gives a very pronounced swollen, 'water-logged' appearance (figs. 5, 8). It is evident that in such a case the weight of the gland might remain normal, even with a marked atrophy of the parenchyma. The fibers adjacent to the follicles may form a denser layer, resembling a basement membrane (fig. 8). The nuclei of the connective tissue cells become shrunken and pyknotic. The vessels may appear nearly normal, although degenerated areas sometimes appear hyperemic.

c. Structure of the thyroid gland in adult rats after acute or chronic inanition

In 6 adult rats whose thyroid was studied the body weight had been reduced 29 to 38 per cent by acute inanition (water but no food) for 8 to 11 days; and in 3 adults the amount of food had been reduced so there was a gradual loss of 33 to 37 per cent in body weight during the chronic inanition period of 5 weeks. The thyroid gland averaged about 0.039 g. (0.24 per cent of the net body weight) in both series, which (compared with controls)

indicates little or no loss in absolute weight during inanition. There was found, however, in a larger series of observations an apparent loss of about 22 per cent in the weight of the thyroid gland during chronic inanition (Jackson '15 a).

In the series studied, the structure of the thyroid varied considerably, but on the whole the changes appear to be essentially similar to those already described for the younger rats held at maintenance (really in a condition of chronic inanition).

No constant difference was apparent between the adults with acute and those with chronic inanition. The thyroid follicles are usually but little if any reduced in size. The colloid usually appears nearly normal in amount and structure, and colloid makes up a large portion of the gland. The cells are frequently cuboidal in the central portion of the gland, but on the average somewhat reduced in height. Also, especially in the few cells not reduced in size, the cytoplasm usually assumes a pronounced coarsely vacuolar structure ('hydropic degeneration'), which probably signifies an increase in water with a reduction in the amount of living substance. The nuclei frequently appear but slightly reduced in size, and show a marked tendency to hyperchromatosis (various stages of pyknosis), more rarely hypochromatosis (karyolysis). In the frequent advanced stages of degeneration, desquamation of the follicular epithelium with obliteration of the follicles occurs as previously described in the normal adult gland (fig. 10). Since these degenerative changes occur also in the normal (control) rats, they could not be considered a result of the experiment, except that they usually appear much more extensive and pronounced in the rats subjected to inanition. In the younger rats, however, as we have seen, the degenerative changes occur much less frequently in the normal rats, and the younger animals are therefore more satisfactory for the experiment.

The stroma of the thyroid in adult rats as a rule shows little change during inanition, although there is some tendency to increase in amount, with slight edema (less marked than in the younger rats). There is also frequently a slight hyperemia, especially in degenerating areas. The increase in the volume of

the interstitial substance is probably sufficient in most cases to offset the decrease in volume of parenchyma, so that if the colloid remains approximately normal in amount, the stationary absolute weight of the adult thyroid gland during inanition is explained. The greater tendency to loss in weight of the thyroid during chronic inanition and in younger rats is probably due to greater atrophy of the parenchyma, and in some cases to loss of colloid.

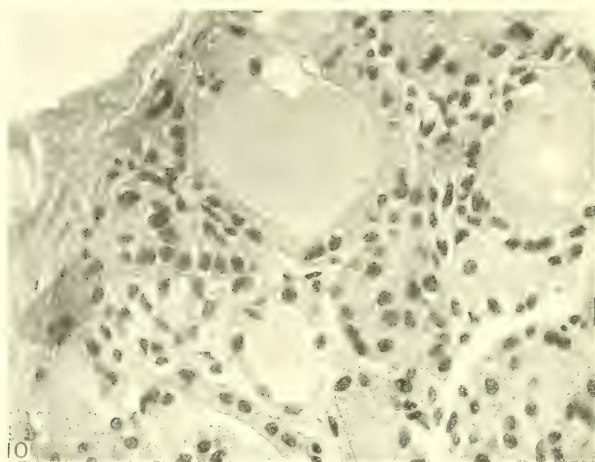


Fig. 10 From a photograph of a portion of the thyroid gland of a normal adult rat (No. S 14 x, gross body-weight 252 grams), moderately magnified to show typical 'spontaneous' degeneration of the follicles. Nuclei in various stages of pyknosis. Tendency to desquamation of epithelium with obliteration of the follicular cavity. ($\times 350$.)

d. Discussion and conclusions

The question of the extent and significance of the normal variation in the thyroid, with especial reference to the so-called degenerative changes, will be considered first, followed by a discussion of the specific changes produced by inanition.

The extreme variability in the form and structure of the follicular epithelium was observed by many of the earlier investigators. These variations were usually ascribed to age differences or to functional changes. The frequent presence of cells

in the follicular cavity was also noted, and the question as to whether the colloid is derived from these desquamated cells or is secreted by the epithelium of the follicular wall was long disputed.

Langendorff ('89), in addition to his well-known 'chief' and 'colloid' cell types, also recognized a retrogressive, degenerative type, which however he did not clearly differentiate from his 'colloid' type (except in theory). Langendorff's results were confirmed by Bozzi ('95), Schmid ('96) and others.

On the other hand, Hürthle ('94) claimed that colloid is formed both by cells of the 'colloid' type and by transformation of desquamated epithelium. Anderson ('94) Müller ('96) and others have opposed the theory that cells of Langendorff's 'colloid' type represent functionally active cells, considering them either functionally exhausted or atrophic, degenerative types, which appears to be the most reasonable interpretation.

De Quervain ('04) made an extensive study of thyroid pathology in man and animals (experiments on dogs and monkeys). From his results, the following conclusions are quoted concerning desquamation and degeneration of the follicular epithelium:

(p. 139) Wir halten also als Ergebnis dieser 3 Versuchsgruppen fest, dass sich sowohl durch Toxinwirkung, wie auch durch Erzeugung von hochgradigen Zirkulationsstörungen an der Schilddrüse histologische Veränderungen erzeugen lassen, die wesentlich durch Wucherung, Desquamation und Degeneration der Epithelzellen gekennzeichnet sind.

(p. 142) Wir kommen zu dem Verhalten der Epithelzellen, das uns vor allem interessiert. Die Reaktion derselben auf jeglichen Reiz besteht hauptsächlich in Desquamation. Wenn wir in diesem Abschnitte von Desquamation reden, so verstehen wir darunter die Abstossung von Zellen mit nachfolgendem Ersatz, und nicht etwa nur die postmortale Ablösung der Epithelzellen von der Bläschenwand. Auch die vitale Ablösung der Zellen ohne Ersatz infolge von Nekrose wird uns nur ausnahmsweise beschäftigen. Die Abstossung von Epithelzellen ist an sich ein normaler Vorgang, und lässt sich an völlig gesunden Schilddrüsen nachweisen; doch ist die normale Abstossung eine sehr beschränkte und man wird nur ab und zu in einem Bläschen eine oder einige Zellen bezw. Kerne in Kolloid schwimmen sehen.

Similar retrogressive changes (frequently considered normal) have been described in the thyroid gland in children, and even

in newborn and late fetuses (Zielinska '94, Elkes '03, Hesselberg '10, Isenschmid '10, Gleim '12).

Watson ('09) in 40 wild rats (species not stated) weighing 60 to 370 grams found wide variations in the thyroid structure, which he described under four types. His first type presents large colloid-filled follicles with flattened cells and darkly-stained nuclei. His second type shows smaller follicles with small cuboidal cells with relatively little cytoplasm and with rounded nuclei (appearing deeply-stained in his figure 2 A). In type 3, the follicles are small, cells large with abundant cytoplasm, and colloid faintly-staining. In type 4, the follicle cells are detached and distorted, with pycnotic nuclei and cytoplasm of variable amount and appearance. Types 1 and 2 (with intermediate forms) predominated in 31 of the 40 glands, type 3 in 5 and type 4 in 4 cases.

Watson concluded that these variations found in thyroid structure "have been induced by dietetic or other factors in the animal's environment." He thought that they did not represent different stages of functional glandular activity (related to digestion), since they were not correlated with the nature of the stomach contents. He does not mention the possibility of inanition, which according to my results might be the cause of his types 1 and 2. The fact that type 1 was found in animals with both full and empty stomachs does not disprove this, as the history previous to capture is unknown and the length of time the animals were fed is not stated. Type 3 is obviously that usually described as normal and type 4 corresponds to the degenerative type which I have found frequently in apparently normal animals.

In previous work, Watson ('05) had studied the effect of a meat diet upon the thyroid gland of 12 tame rats (11 were 6 to 12 weeks of age, 1 adult) with 8 controls. The experiment lasted 6 weeks to 4 months; and the meat-fed animals were usually retarded in body weight. Ten of the 12 showed marked changes in the thyroid, including congestion, epithelial proliferation of the follicles and degeneration of the colloid. (Similar results in meat-fed rats were obtained by Tanberg, cited by Biedl '13.)

In a later paper, Watson ('07 a) studied the thyroid in 20 wild rats, 10 of which were killed immediately after capture and 10 fed bread and milk for 10 weeks. The former showed large colloid-filled follicles with flattened epithelium; the latter had smaller follicles, with little colloid and cubical epithelium. Waters ascribes the change to the difference in diet; but here, as also in his meat-fed rats, it is difficult to exclude the factor of inanition (inadequate nutrition). He also failed to note that the degeneration may occur apparently spontaneously. In another paper Watson ('07 b) finds in young rats fed on oatmeal diet for several weeks a marked enlargement of the thyroid (0.078 per cent of the body weight as compared with 0.029 per cent in controls). In some cases, the thyroid epithelial cells were swollen and detached.

Rebello and DaCosta ('10) describe the thyroid of the normal rabbit as variable in structure with the occurrence of epithelial desquamation and degeneration, resulting in obliteration of the follicles in a manner very similar to that described above for the rat. They consider this a normal physiological process following functional activity. However they find the degeneration markedly increased by the injection of thyroid-proteid extracts. They also observed similar degenerated areas in the thyroid of a normal young dog.

Douglas ('15) in an extensive study of the effects of various diets upon the thyroid of pigeons, chicks and rats, described four types of structure, somewhat similar to those of Watson. He concludes that:

The histological appearances do not represent different stages of secretion, comparable to those of secreting glands engaged in the process of digestion. Under similar conditions and in animals fed on similar diets, the appearances in the thyroid differ very markedly. One observes all stages from the type with large vesicles, full of colloid with flattened cells, to that of a thyroid with no colloid and columnar shaped cells. Also the thyroid may be wholly or partly disintegrated. The variation in appearance of the thyroid seems to depend to some extent on the nutrition, and is thus only in this way dependent on the diet.

Out of 31 rats (species not stated) with "ordinary laboratory diet of bread" Douglas found the degenerative type of thyroid

only once, though it occurred frequently in pigeons and chicks. It occurred very frequently (8 of 11 cases), however, among rats fed various food mixtures with fat. The normal rats usually presented thyroid follicles with cubical cells and a moderate amount of colloid.

The variability in the structure of the thyroid gland and its physiological and pathological relations have been discussed by Marine and Lenhart ('09 a, '09 b, '11 a, '11 b, '11 c) in a series of papers. On the basis of an extensive investigation of the human and animal (dog, sheep, ox, pig, etc.) thyroid, they distinguish as 'physiological' the following: (1) normal resting gland, with low cuboidal epithelium; (2) hyperactive stage, with hyperemia, cells becoming hyperplastic and columnar, colloid disappearing; (3) colloidal stage (of recovery), in which conditions again return to (1) with abundant colloid. If hyperactivity continues without rest, however, colloid disappears and the cells die of exhaustion, becoming progressively degenerated and desquamated with varied degrees of disintegration. The nuclei in this case are described as enlarged, often hyperchromatic, and quite variable in appearance. Similar desquamation and degeneration of the follicular epithelium is described in normal senile atrophy. The flattened type of epithelium, indicating a minimum functional activity, may be experimentally produced by administration of iodine in the diet; whereas a diet without iodine induces the columnar, hyperactive type of thyroid cells.

The variability in structure due to the extremely sensitive nature of the thyroid gland is emphasized by Marine and Lenhart ('11 c) as follows:

It has long been recognized and frequently emphasized by us that the thyroid tissue is extremely labile—reacting quickly to relatively slight physiological variations in the body metabolism, and for this reason may show even daily histological changes within narrow limits.

Marine ('15) also notes that thyroid glands undergo autolysis in a few hours after removal from the body, especially if kept near the body temperature, resulting in desquamation of the alveolar epithelium.

In a recent communication in reply to a letter of inquiry by the author, Dr. Marine states that he has frequently observed the so-called degenerative changes in the thyroid of the rat and other animals, and comments as follows:

I personally consider this change as a kind of autolysis. Autolysis in the thyroid is perhaps more easily recognized than in any other organ of the body because of its simple architecture. The change of which you speak consists of a desquamation of the alveolar cells and a breaking up of the cell membrane, giving a ragged appearance to the free border of the cells. This is always more marked in the slightly hyperplastic stages. Much has been made of this by various experimenters in an attempt to bring it into relation with the specific experiments that they were studying. It is of too general occurrence to have so varied a significance. The rat and the chick we have found to show these changes most frequently. One sees it in all chicks or rats that are autopsied some hours after death and even in rats sacrificed and autopsied at once, as all ours have been just for the purpose of eliminating this autolysis. I also think that formalin fixation is favorable for its development, as one rarely sees it in strictly fresh thyroids that have been fixed with metallic salts. In human thyroid pathology, as you are aware, this change has been described in almost every paper and in connection with the Basedow syndrome. Most writers who have studied the human thyroid have attempted to ascribe some peculiar toxic value to it. This is wrong. It is present in the cretin thyroid or in any active hyperplastic thyroid as well. . . . In conclusion, therefore, I believe that this change is quite common in rats and fowl thyroids generally, and is of frequent occurrence in all thyroids unless the special precaution is taken of obtaining the tissues strictly fresh and fixing them in solutions other than formalin. I do not think it is any more common in starvation experiments than in other groups.

It might also be added that glands which have not been placed promptly in the fixative, and in which the process of post mortem autolysis has begun, sometimes show considerable differences in appearance at different depths from the surface. The fixation is better in the superficial layers than in the deeper strata which require a longer time for penetration.

In order to compare the structure of the thyroid gland, and especially the occurrence of degeneration, in the rats used by me with those elsewhere, I have obtained material from various localities. Professor Addison, of the University of Pennsylvania, kindly furnished mounted specimens of the thyroid from 3 nor-

mal albino rats. In a young rat (121 days), the structure was found nearly normal, the epithelial nuclei rarely showing incipient pycnosis. In 2 older rats, however, (200 and 452 days), there was found a considerable amount of epithelium showing desquamation and typical degenerative changes. In the thyroid of a gray rat (said to be a hybrid gray-albino) sent by Professor Evans, of the University of California, the structure was found nearly normal, but a few areas showed typical degeneration with partial desquamation of the epithelium.

Through the courtesy of Professor Bensley and Mr. Burgett, I obtained mounted specimens from 8 thyroids of normal albino rats (body weight 117 g. to 287 g.) from the University of Chicago laboratories. Of the 8 glands, 2 showed extensive follicular degeneration, 1 moderately extensive and 2 very small areas of slight degeneration. Only 3 appeared perfectly normal throughout.

Concerning the degenerative appearances found in the thyroid gland, Dr. Bensley writes:

There are two circumstances which should be borne in mind in connection with these changes in the thyroid gland, which frequently, I think, are responsible for similar changes in tissues which were normal to start with, namely: drying of the surface of the gland when adequate care is not taken in transferring to weighing bottles; and pinching with forceps in the process of extraction. The latter frequently initiates a rapid change in the cell which is marked by acid reaction to indicators, and by greater affinity for basic stains in the fixed preparation, with loss of characteristic structure by the nucleus.

In connection with the suggestion by Dr. Bensley of the importance of traumatic injury during the removal of the organ in producing degenerative changes, the observations of Sutherland ('15) upon pycnotic changes in nuclei of the spinal cord near the seat of injury may be cited.

From the foregoing, it appears that the characteristic follicular degeneration of the thyroid appears to a variable extent in apparently normal rats, both wild and albino, in widely separated localities.

Summing up the literature cited, together with my own observations, we may conclude that the thyroid gland shows an

unusual amount of normal variability in histological structure, doubtless due at least in part to functional stages which are as yet imperfectly understood. Furthermore, on account of the extreme sensitiveness of the thyroid, it reacts strongly to various influences by changes which lie on the border-line between normal and pathological. So-called degenerative conditions (desquamation and degeneration of epithelium) appear to a limited extent even in the thyroids of normal animals (including man), although these conditions probably have no physiological significance. Under various abnormal conditions, these retrogressive changes appear to be increased in extent and intensity.

Having reviewed the variable structure of the thyroid, we may now consider briefly the specific effects of inanition, as found by the few investigators who have studied the histological changes.

Barbèra ('02) studied the effects of acute inanition upon the thyroid gland in 3 rabbits and 1 dog, with controls of the same sex and from the same mother. The rabbits were starved 7 to 11 days, and the dog 21 days, with loss of about 30 per cent in body weight in each case. No data are given as to weight of the gland. No measurements of the entire cells are stated, but they were found reduced in size, the loss in the cytoplasm being greater than in the nucleus. The average of a large number of measurements of the nuclear diameters showed in the rabbit 5.73×4.99 micra in the controls, and 5.75×3.84 in the starved animals. This would indicate a reduction in size with relative elongation of the nuclei. This elongation of the nucleus was not found in the dog, however, where the average of 5.11×4.62 micra in the controls was reduced to 4.44×4.28 micra in the starved. The intercellular substance was also found reduced in amount, but colloid formation appeared to continue normally (hence no symptoms of hypothyroidism).

As above stated, I have likewise found in the rat thyroid during inanition a slight reduction in the size of the nucleus, which is usually much less marked than the decrease in cytoplasm. I find the shape of the nucleus dependent upon the form of the cell; in cases where the cell becomes flattened, the nucleus becomes correspondingly elongated, but otherwise it usually re-

tains its spherical or ovoidal (ellipsoidal) form. Morgulis ('11) found a tendency to relative elongation in the nuclei of various organs in *Diemyctylus* during starvation, but not in the liver and pancreas of the albino rat.

Traina ('04) finds in the thyroid of the rabbit during inanition a decrease of about 30 per cent in the volume of the cells, the loss being relatively greater in the cytoplasm than in the nucleus. The cells lose more in height than in breadth; the nucleus remains distinct and regular in outline. The fatty granules (previously described in the thyroid by Erdheim '03) remain in the atrophic cells unchanged in position, number, form and size.

Missiroli ('11) in rabbits finds the thyroid structure correlated with the stage of digestion present. When food is withheld, the colloid is no longer eliminated but accumulates and distends the follicles. In some cases of prolonged fasting, the colloid may undergo 'fatty degeneration.' In advanced stages of inanition, nuclei and protoplasm undergo atrophy, and the interstitial (connective) tissue appears increased. On refeeding, the colloid accumulated in the follicles is rapidly eliminated.

Mrs. Thompson ('11) states that:

In a dog which had been subjected to a few days' inanition, the thyroid (hardened in strong Flemming's fluid) appears very different from the normal. The cells appear swollen rather than proliferated, and in some cases vesicles are filled with cells. The vesicles are shrunk so as to assume various shapes, and much intervesicular material appears. The general appearance is as if the gland had been roughly squeezed, so that the vesicles are any shape but spherical. The change wrought by inanition seems to make the structure of the gland tend towards that of the parathyroid.

It is doubtful to what extent the changes described by Mrs. Thompson were actually due to inanition, since the inanition period was relatively short (for a dog) and the normal variation in structure is uncertain. She describes a similar appearance in the thyroid of a cereal-fed dog, while that of a meat-fed dog was normal.

In general, therefore, we find that the observations upon the structure of the thyroid during inanition, including those of pre-

vious observers and my own, while differing in various minor details, agree in the main. The epithelium of the thyroid follicles apparently undergoes at first a simple atrophy, which affects the cytoplasm more than the nucleus. The cells thus become reduced in height, with relatively large nuclei. In advanced stages of inanition, degenerative phenomena (found also to a limited extent even in the normal gland) become increasingly evident. The follicular cells may remain in situ, but usually are desquamated, replacing the colloid (which otherwise remains nearly normal). The cytoplasm, typically vacuolated in the earlier stages, may become collapsed, deeply-staining and more or less homogeneous ('colloid' type) in appearance, or may disintegrate, forming an irregular granular mass. The nucleus usually becomes hyperchromatic, undergoing successive stages of karyopycnosis, ending in extreme cases in karyorrhexis. Sometimes, however, it becomes hypochromatic, and undergoes karyolytic changes.

It is somewhat remarkable that these characteristic changes during inanition,—cell-atrophy with characteristic, more or less extensive nuclear and cytoplasmic degeneration,—are likewise found to a greater or less extent associated with a wide range of other conditions. In the foregoing pages, changes in thyroid structure somewhat similar to those associated with the various degrees of inanition have been mentioned in connection with the following:

1. Spontaneous (?) degeneration (in normal animals without apparent cause).
2. Age changes (tendency of cells to become flattened with age; senile atrophic changes).
3. Functional changes (varied changes due to functional activity; atrophy in the exhaustion following hyperactivity).
4. Changes due to effects of diet (meat and other special diets; administration of iodine).
5. Toxic effects (changes produced by injection of various toxic substances, proteid extracts, etc.).
6. Circulatory disturbances (stasis produced by ligation of vessels, etc.).

7. Pathological conditions (thyroiditis, exophthalmic goiter, etc.), probably due to infections.

8. Results of imperfect fixation.

9. Post mortem changes (due to mechanical injury, post mortem autolysis, etc.).

To this should be added the statement that very similar cell-changes have been described, especially by pathologists, in various tissues of the body, most frequently in the epithelia of the various parenchymatous glands. Changes in the renal epithelium following ligation of vessels, infarcts, intoxications, infections, post mortem changes, etc., form a familiar example, in which the phenomena are in many respects strikingly similar to the degenerative changes above described for the thyroid gland.

The remarkable similarity in the degenerative changes thus found in various organs and under apparently widely different conditions naturally raises the question as to whether the underlying fundamental process may not be at least in some respects essentially similar in all cases. It may be possible, for example, that cellular inanition may be produced in different ways, and may therefore be the essential factor in the process of degeneration arising from varied causes. If, as is now generally assumed, cellular metabolism is accomplished by means of numerous varieties of intracellular enzymes, one could easily understand how anything which would destroy or interfere with the activity of any of the enzymes necessary to anabolism might thereby produce a condition of cellular inanition, even in the presence of an abundant food-supply. The probable importance of autolytic enzymes in the phenomena of cell-degeneration, especially in the nuclear changes of pyknosis, karyolysis, etc., has been emphasized by Wells ('14) and others. A final solution of the problem is impossible at the present time, on account of our scanty knowledge of normal cell-physiology; but it seems probable that many of the phenomena of cellular degeneration from various apparent causes will ultimately be explainable as due essentially to cell-inanition.

THE PARATHYROID GLAND

The normal structure of the parathyroid glands will be considered first, followed by a discussion of the changes produced during inanition.

a. Normal structure of the parathyroid glands

As is well known, there is in the rat a single pair of parathyroid glands, said to correspond to parathyroid III. In one exceptional case, I found a parathyroid (see table 1) on one side only. I have never observed the accessory parathyroids described by Erdheim (cited by Biedl '13). The parathyroids are located upon the external surface of each lateral lobe of the thyroid gland, usually somewhat posteriorly, though occasionally more anteriorly (figs. 1, 3, 4, 5, 6).

Ochsner and Thompson ('10) state that the parathyroids of the rat are located within the thyroid lobes near the upper pole. I find, however, that there is considerable variation; they occur slightly more frequently opposite the middle third of the thyroid lobe, but often at more cephalic or caudal levels. Of 59 glands whose position was noted, 18 were located in the cephalic third of the thyroid, 20 in the middle third, 13 in the caudal third, 6 at the junction of the cephalic and middle thirds, and 2 at the junction of middle and caudal thirds. The parathyroids of the two sides are usually, but not always, at nearly the same level. In 3 cases observed, one parathyroid was located in the cephalic third of the lateral lobe of the thyroid, while the opposite parathyroid corresponded to the caudal third of the thyroid.

The parathyroid gland lies partly embedded within the thyroid, about one-fourth to one-third of its surface usually forming an external free or exposed surface (figs. 1, 3, 4, 5, 6). Sometimes it may project more extensively from the thyroid surface; in other cases it lies more deeply, rarely entirely embedded within the thyroid.

As to size, the dimensions of the parathyroid gland at various ages are given in the following table.

TABLE I

Dimensions of parathyroid glands in albino rats. Measurements from mounted sections

| RAT NO. AND SEX | AGE | BODY-LENGTH | BODY-WEIGHT GROSS (AND NET) | DIMENSIONS OF PARATHYROIDS | | AVER. DIAM. |
|---------------------------------|------|-------------|-----------------------------|----------------------------|------------------|-------------|
| | | | | a | b | |
| | days | mm. | grams | mm. | mm. | mm. |
| <i>A. Normal (control) rats</i> | | | | | | |
| St 32.1 f | 3 | 46 | 5.0(—) | 0.22×0.18×0.26 | 0.26×0.18×0.26 | 0.23 |
| V 2.1 m | 14 | 89 | 22.7(—) | 0.34×0.18×0.30 | 0.34×0.18×0.36 | 0.28 |
| S 9.47 f | 22 | 95 | 25.5(23.3) | 0.33×0.32×0.38 | 0.28×0.38×0.38 | 0.35 |
| St 32.2 m | 21 | | 27.2(—) | 0.46×0.32×0.48 | 0.53×0.36×0.50 | 0.44 |
| St 5.1 f | 56 | | 63.0(—) | 0.62×0.56×0.62 | 0.56×0.50×0.52 | 0.56 |
| St 12.54 f | 110 | 166 | 121 (116) | 0.96×0.58×0.72 | 0.83×0.64×0.72 | 0.76 |
| S 5.4 f | 74 | 168 | 126 (118) | 0.64×0.50×0.40 | 0.54×0.50×0.36 | 0.49 |
| St 10.24 f | 117 | 169 | 135 (129) | 1.21×0.65×0.90 | 0.94×0.70×0.90 | 0.88 |
| H 24.3 f | 232 | 188 | 143 (138) | 0.66×0.56×0.88 | 0.50×0.56×0.72 | 0.64 |
| H 65.7 f | 98 | 179 | 144 (139) | 0.57×0.43×0.64 | 0.55×0.40×0.80 | 0.57 |
| H 60.3 f | 78 | 182 | 146 (138) | 0.47×0.58×0.74 | 0.75×0.48×0.74 | 0.63 |
| H 68.3 f | 74 | 185 | 158 (151) | 0.45×0.48×0.58 | 0.46×0.48×0.52 | 0.50 |
| H 42.3 f | 182 | 195 | 170 (160) | 0.55×0.64×0.74 | 0.78×0.60×0.70 | 0.67 |
| S 5.3 m | 74 | 180 | 172 (167) | 0.62×0.36×0.47 | (not observed) | 0.48 |
| H 47.3 m | 145 | 201 | 174 (168) | 0.54×0.64×0.72 | 0.66×0.54×0.80 | 0.65 |
| H 27.3 f | 253 | 195 | 175 (166) | 0.84×0.58×0.90 | 0.89×0.60×0.80 | 0.77 |
| MI 2 m | 74 | 208 | 181 (173) | 0.49×0.58×0.74 | 0.56×0.46×0.67 | 0.58 |
| H 34.3 f | 224 | 194 | 181 (173) | 0.70×0.54×1.12 | (1 gland absent) | 0.79 |
| H 34.6 f | 225 | 205 | 194 (188) | 0.57×0.70×0.86 | 0.84×0.56×0.76 | 0.71 |
| S 33.116 f | 349 | 195 | 194 (188) | 0.79×0.60×0.86 | 0.90×0.68×0.70 | 0.76 |
| H 70.3 m | 70 | 194 | 208 (198) | 0.63×0.48×0.80 | 0.79×0.53×0.80 | 0.67 |
| H 27.6 m | 254 | 214 | 229 (222) | 0.75×0.75×0.85 | 0.88×0.60×0.80 | 0.77 |
| H 50.3 m | 141 | 211 | 230 (222) | 0.75×0.60×0.90 | 0.77×0.72×0.90 | 0.77 |
| S 14 x m | ? | 205 | 252 (247) | 0.68×0.55×0.65 | 0.72×0.55×0.80 | 0.66 |
| H 54.3 m | 101 | 222 | 284 (275) | 0.86×0.60×0.80 | 0.66×0.64×0.80 | 0.73 |
| St 14 m | 442 | 239 | 322 (314) | 0.88×0.72×0.96 | 1.07×0.85×0.90 | 0.90 |

B. Rats at maintenance from 3 to 10 weeks of age

| | | | | | | |
|-----------|----|-----|------------|----------------|----------------|------|
| S 5.10 f | 67 | 100 | 22.7(21.0) | 0.49×0.40×0.42 | 0.52×0.37×0.50 | 0.45 |
| S 11.63 f | 72 | 95 | 23.8(22.6) | 0.50×0.50×0.50 | (not observed) | 0.50 |
| S 11.65 m | 73 | 100 | 23.8(22.5) | 0.48×0.44×0.53 | 0.47×0.48×0.52 | 0.49 |
| S 11.64 f | 73 | 100 | 24.2(22.9) | 0.61×0.55×0.65 | 0.68×0.50×0.60 | 0.60 |
| S 12.69 f | 66 | 100 | 24.5(22.7) | 0.43×0.50×0.65 | 0.46×0.55×0.55 | 0.52 |
| S 6.23 f | 72 | 102 | 26.1(24.5) | 0.72×0.40×0.30 | (not observed) | 0.47 |
| S 5.8 f | 73 | 105 | 27.6(24.6) | 0.50×0.45×0.35 | 0.50×0.45×0.35 | 0.43 |

TABLE 1—Continued

| RAT NO. AND SEX | AGE | BODY- LENGTH | BODY-WEIGHT GROSS (AND NET) | DIMENSIONS OF PARATHYROIDES | | AVER. DIAM. |
|---|------|-----------------|--------------------------------|-----------------------------|----------------|----------------|
| | | | | a | b | |
| | days | mm. | grams | mm. | mm. | mm. |
| <i>C. Adult rats with acute inanition¹</i> | | | | | | |
| M 1 m | (?) | 205 | 248 to 168(165) | 0.50×0.60×0.80 | 0.45×0.64×0.80 | 0.63 |
| M 2 m | (?) | (?) | 254 to 170(167) | 0.69×0.48×0.80 | 0.57×0.52×0.80 | 0.64 |
| S 16 f | (?) | 195 | 267 to 190(186) | 0.75×0.51×0.52 | 0.78×0.50×0.55 | 0.60 |
| S 27 m | (?) | 215 | 320 to 223(219) | 0.77×0.60×0.80 | 0.85×0.60×0.80 | 0.74 |
| S 25 m | (?) | 205 | 328 to 202(198) | 0.73×0.52×0.70 | 0.88×0.58×0.66 | 0.68 |
| <i>D. Adult rats with chronic inanition¹</i> | | | | | | |
| M 3 m | (?) | 175 | 188 to 125(122) | 0.48×0.48×0.96 | 0.49×0.66×0.72 | 0.63 |
| M 6 m | (?) | 175 | 220 to 138(134) | 0.35×0.45×0.70 | 0.35×0.70×0.75 | 0.55 |
| M 11 m | (?) | 190 | 264 to 163(158) | 0.55×0.52×0.72 | 0.52×0.50×0.70 | 0.59 |

¹ Both initial and final body weights given.

In the first column, the letters 'St', 'V,' etc., refer to different series of rats used. The number preceding the decimal point is the litter number; the number following is the individual number. 'm' refers to male, 'f' to female. Under 'Dimensions of parathyroids,' the measurements for the two glands are listed separately in columns 'a' and 'b'.

The cephalo-caudal length (which is always the first of the three dimensions given in the table) was calculated from the number of sections, of known thickness, through which the gland extended. The other dimensions represent the largest and smallest diameters measured in the largest cross-section of the gland, in the mounted sections. It should be noted, therefore, that these dimensions are smaller than in the fresh gland, as each linear dimension has probably undergone a shrinkage of at least 10 per cent in the process of fixation, dehydration and embedding.

Generally speaking, the parathyroid presents a slightly flattened, ellipsoidal form, the relative dimensions usually corresponding in a general way to those of the associated lobe of the thyroid. That is, in cross-section the outline usually appears somewhat compressed medio-laterally; but the cephalo-caudal

dimension is greatest in less than half of the cases. It will be noted, however, that the parathyroid is frequently nearly spherical in form, the three axes being approximately equal (table 1: figs. 1 and 3 to 6).

The normal growth in the dimensions of the parathyroid is evident from the table. In the pair of newborn parathyroids, the average diameter is 0.23 mm.; at 2 weeks it is 0.28 mm.; at 3 weeks 0.35 to 0.44 mm.; and at 8 weeks, 0.56 mm. In 5 rats from 70 to 74 days of age, the average is 0.54 mm. (range 0.48 to 0.67 mm.). The dimensions increase very irregularly up to 0.90 mm. average in a rat of 322 g. gross body weight, 442 days of age.

In spite of the comparatively small number of observations and the admitted inadequacy of the average diameter as a measure of the size of the parathyroid, it is believed that these data are useful for purposes of general comparison. The data for the individual parathyroids were compared with the weights of the corresponding thyroids (not shown in the table), but there is apparently no close correlation between them.

A point of special interest, which is not apparent from casual inspection of the table, appears more clearly when the observations are plotted graphically according to the body weight and average diameter of the parathyroid. The gland appears to be relatively larger in the females. Further observations will of course be necessary to establish this point with certainty; but if true, this will place the parathyroid in a class with the hypophysis and suprarenals, in which the female rats possess relatively larger glands.

The normal structure of the parathyroid gland in the albino rat at 3 weeks of age is shown in figure 11. The epithelial cells are grouped in irregular, closely-packed masses, occasionally showing a cylindrical or cord-like arrangement.

In size the parathyroid epithelial cells vary considerably, the average being about 6 x 8 micra, or slightly larger. They are thus considerably smaller than the thyroid epithelium of a corresponding gland. The cell boundaries are usually distinct.

The cytoplasm is relatively small in amount, forming a narrow zone 1 to 2 micra in width around the nucleus. The cytoplasm is usually finely granular and pale reddish-violet in appearance (with Zenker fixation and haematoxylin-eosin stain). No 'oxyphile' (eosinophile) cells are present.

The nuclei of the parathyroid epithelium (fig. 11) vary considerably. They usually appear elongated, the smaller rounded forms perhaps representing cross-sections of ellipsoidal nuclei. There is considerable variation in size (average about 4 x 6 micra) and form. The nuclear outline frequently presents a notched or lobulated appearance.

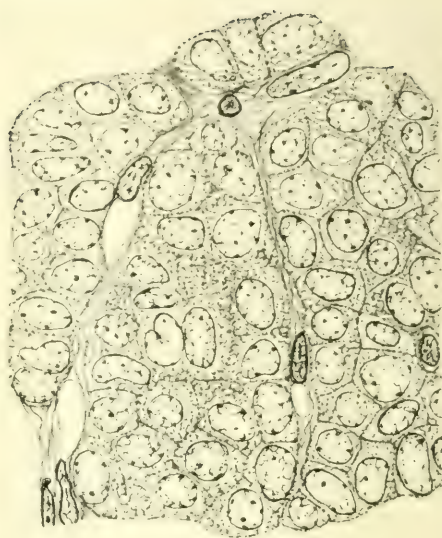
In structure, the parathyroid nuclei are similar to those of the thyroid, presenting a distinct, deeply-staining nuclear membrane, and a faint nuclear network with usually one or two distinct nucleoli (karyosomes), in a clear, light, homogeneous background corresponding to the nuclear sap (karyolymph). Mitoses are relatively abundant at this stage, two or three being found in each cross-section near the center of the gland.

The parathyroid gland, like the thyroid, is in general fairly uniform throughout in structure at this time, but certain irregularities may appear, even at this age. In the most superficial stratum of the gland, especially on the external free surface (not in contact with thyroid) modified cells may appear. These cells usually form small groups of somewhat atrophic appearance. The cytoplasm is more irregular, vacuolated or denser and deeply-staining. The nuclei of these cells are generally somewhat smaller than usual, often flattened, and with more or less deeply-staining homogeneous appearance characteristic of the earlier stages of pycnosis. Such atrophic cells rarely occur except near the surface, and perhaps represent a mild form of pressure-atrophy (as found also near the surface in the thyroid).

The interstitial connective tissue forms a delicate, fibrous stroma, like that of the thyroid, except that it is usually not so highly vascular (fig. 11). In a few places, it is somewhat more abundant, forming scattered, irregular, light streaks observed in the interior of stained cross-sections. These streaks apparently become more distinct in the older rats.



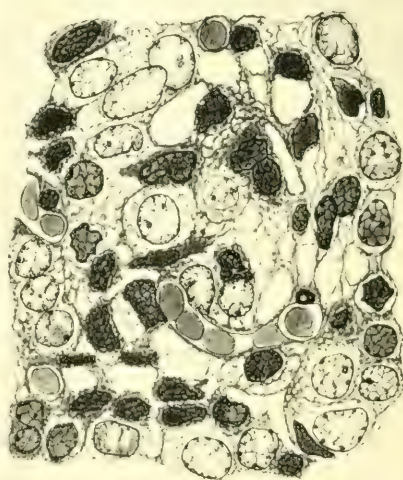
11



12



13



14

In the parathyroid at 10 weeks, the structure is essentially the same as at 3 weeks (fig. 11), no important change in the appearance or average size of the cells being observed. Mitoses appear somewhat less frequently, only 1 or 2 in a section. The atrophic or degenerated types appear usually somewhat more frequently and more pronounced in character, however. They most commonly appear in the superficial stratum, especially at the exposed surface (not in contact with thyroid); but occasionally in the interior also, usually in scattered areas. The nuclei of these cells are usually deeply-staining and pyknotic, though vesicular karyolytic forms also occur. The cytoplasm in these atrophic types is usually rarefied and vacuolated in appearance, but occasionally somewhat coarsely granular and deeply-staining (eosinophile), suggesting in some respects the 'oxyphile' type described as normal by some authors for the

Fig. 11 A small portion of the parathyroid gland shown in figure 1 (rat S 9.47, normal 3 weeks), magnified to represent the details of the normal histological structure (which is essentially the same as at 10 weeks). Epithelial cells in irregular masses; cytoplasm granular, scanty in amount; nuclei irregularly elongated in appearance. Stroma scanty, vascular. ($\times 750$.)

Fig. 12 A small portion of the parathyroid gland shown in figure 4 (rat No. S 11.63, held at maintenance from 21 to 72 days of age), magnified to show details of histological structure. This area represents the hypochromatic (incipient karyolytic) type, which is relatively infrequent. The nuclei appear light and swollen. The cytoplasm here is apparently not reduced in amount, but shows tendency to vacuolization and disappearance of the normal granulation. Stroma normal. ($\times 750$.)

Fig. 13 A small portion of the parathyroid gland shown in figure 5 (rat No. S 5.10, held at maintenance from 21 to 67 days of age), magnified to show the details of histological structure. This area represents the typical hyperchromatic type (various stages of karyopycnosis). Cytoplasm more or less reduced in amount, with marked vacuolization ('hydropic degeneration'). Stroma normal. ($\times 750$.)

Fig. 14 A small portion of the parathyroid gland shown in figure 6 (rat No. S 11.64, held at maintenance from 21 to 73 days of age), magnified to show the details of histological structure. This area represents a somewhat advanced stage of degeneration, which frequently involves small, scattered masses, but is rarely extensive. The nuclei are partly of the hypochromatic (karyolytic), and partly of the hyperchromatic (karyopyknotic) type. Around the former, the cytoplasm is usually vacuolated ('hydropic degeneration'); while around the latter type cytoplasm appears reduced in amount, deeply-staining (eosinophile) and frequently coarsely granular in appearance. ($\times 750$.)

parathyroid of various animals (including man). Sometimes spaces are seen between the larger bundles of connective tissue and the adjacent parenchyma. These spaces somewhat resemble in appearance the paratrabecular and subcapsular lymph-sinuses in lymph-nodes.

In the older and adult animals, the parathyroid gland shows essentially the same structure as described at 10 weeks. The cells average perhaps slightly larger, the nuclei being typically elliptical and averaging between 4×6 and 5×7 micra. The cytoplasm forms a light granular zone 1 to 2 micra in width. Mitoses are not found in the adult rats. The atrophic or degenerative types of cells occur frequently, though usually in limited, scattered areas, especially near the exposed surface of the parathyroid.

b. Structure of the parathyroid glands in young rats held at maintenance

If in the rats held at maintenance (constant body-weight) from the age of 3 to 10 weeks the dimensions of the parathyroid glands be compared with those of the controls at 3 and 10 weeks (table 1), it appears that the parathyroid has not shrunk in size during the experiment. On the contrary it has even apparently increased so that the dimensions are nearly as large as those of the full-fed controls of the same age but several times larger in body-weight. As shown in table 1, the average diameter of the normal parathyroid at 3 weeks is about 0.40 mm., at 10 weeks, 0.54 mm.; in maintenance 3 to 10 weeks, 0.49 mm. Thus the parathyroid in young rats held at maintenance becomes relatively very large in comparison with the thyroid (which usually decreases somewhat), as is apparent upon comparison of the parathyroids in figures 1 and 3 with those in figures 4, 5 and 6.

The changes in the histological structure of the parathyroid gland during inanition are on the whole less marked than in the thyroid gland, although in most respects similar in character. In the parathyroids of rats held at maintenance from the age of

3 to 10 weeks, the epithelial cells show a variety of changes in structure, but it is doubtful whether there is in most cases any marked decrease in the average volume of the cells.

The cytoplasm, however, has undergone structural changes similar to those described for the thyroid cells (though somewhat less marked), its finely granular structure usually showing more or less marked vacuolization or 'hydropic degeneration' (figs. 12, 13, 14). In some cases, however, especially with advanced karyopycnosis, the cytoplasm may appear somewhat diminished in amount, and with deeply-staining (eosinophile), coarsely granular structure. The cell-boundaries sometimes remain distinct (fig. 12), but are frequently indistinct (figs. 13 and 14).

Two distinct types of nuclear change occur (similar to those found in the thyroid). In the first or hypochromatic type (fig. 12) the nuclei appear vesicular, swollen and very lightly stained. This type is relatively less frequent (as in the thyroid) and may appear in all the cells over considerable areas (as in fig. 12), or in scattered cells or small groups (as in fig. 14). This type of nucleus probably represents an early stage of karyolysis, though it is doubtful whether any nuclei actually reach dissolution in the specimens studied.

As in the thyroid, the more frequent type of nuclear change in the parathyroid in young rats held at maintenance is in the direction of hyperchromatosis, representing various stages of karyopycnosis (figs. 13 and 14). For the most part, however, the parathyroid nuclei do not go beyond the earlier stages, merely showing a homogeneous background more deeply colored so as to obscure the internal nuclear structure. In these nuclei, there is little if any decrease in size, and this type frequently occurs throughout practically the entire gland. Less frequently more advanced stages of pycnosis are found, in which the nuclei are diminished in size, with homogeneous deep stain (fig. 13). The hyperchromatic and hypochromatic types of cell-degeneration may sometimes occur intermingled, as in figure 14.

No advanced stages involving karyorrhexis were found. Neither mitosis nor amitosis was observed in any case.

The interstitial tissue or stroma of the parathyroid rarely shows any noteworthy changes. In some cases it appears slightly increased in amount, with edemic appearance, but not so often as in the thyroid.

c. Structure of parathyroid in adult rats after acute and chronic inanition

In adult rats subjected to acute or chronic inanition, the dimensions of the parathyroid gland are given in table 1. While it is impossible to draw any final conclusion, on account of individual variation and the small number of observations, there seems to be a decrease in the size of the parathyroid as a result of inanition. When the average diameters and corresponding (final) body-weights are plotted graphically, the parathyroids appear approximately normal. Their decrease during inanition therefore would appear to be nearly proportional to that of the whole body.

In histological structure, the parathyroids in adult rats after acute and chronic inanition show changes essentially like those described for the younger maintenance rats. The changes are in general less marked than in the thyroid, but there is considerable individual variation. Although there is cell-shrinkage in some places, especially where degeneration is marked, in other places there appears no decrease in the average size of the parathyroid cells.

The cytoplasm in adult parathyroids following inanition sometimes appears nearly normal, but usually shows typical vacuolization ('hydropic degeneration'), or in some cases a more deeply-staining homogeneous or coarsely granular structure (especially accompanying markedly pycnotic nuclei).

The nuclei may be nearly normal, but usually show hyperchromatic changes (more rarely hypochromatic), being usually found in the earlier (rarely later) stages of karyopycnosis. These changes occur not merely in restricted areas, or near the free surface (as found in the normal gland) but usually and typically involve large areas and frequently the entire gland. Thus while

no new types of cells occur, the atrophic types which occur infrequently and in small amount in the normal gland become very frequent and extensive during inanition. The changes in general appear to be somewhat more marked and extensive during chronic than during acute inanition, although there are individual variations.

The interstitial connective tissue or stroma of the parathyroid appears usually somewhat more distinct, and often relatively increased slightly in amount, during acute and chronic inanition of adults.

d. Discussion and conclusions

Broadly speaking, we may conclude from the foregoing that in general the effects of inanition upon the parathyroid gland are similar to those on the thyroid, but somewhat less marked. The similarities and differences include the following.

The parathyroid gland apparently belongs to that group of organs which in young rats tend to continue growth, even when the body-weight is held constant. This group includes the eye-balls, spinal cord, testes, skeleton, alimentary canal, suprarenal glands and hypophysis. The thyroid, on the contrary, belongs to the group losing weight (Jackson '15 b).

In the parathyroid, many of the individual cells apparently do not decrease in size during inanition; whereas in the thyroid the decrease is usually well marked, especially in the cytoplasm.

In both parathyroid and thyroid glands, the cytoplasm usually shows marked changes during inanition, whether or not it is decreased in amount. Vacuolization ('hydropic degeneration') is most frequent, but deeply-staining, eosinophile condensations of homogeneous (especially in the thyroid) or coarsely granular (especially in the parathyroid) types may occur.

The nucleus in both parathyroid and thyroid glands during inanition tends to become hyperchromatic (more rarely hypochromatic). Thus the nuclei are commonly found in various stages of pycnosis, the earlier stages being typical in the parathyroid, while in the thyroid the later stages (and even karyorrhexis) may frequently occur.

In both parathyroid and thyroid (especially the latter) the normal structure is somewhat variable, and a few cells of atrophic, degenerative character occur. These atrophic types become much more pronounced and numerous during inanition.

The fibrous interstitial tissue (stroma) undergoes little change during inanition, though sometimes it is increased in amount by edemic infiltration (especially in the thyroid).

Very little is found in the literature concerning the effect of inanition upon the parathyroid glands. Erdheim ('03) finds in the human parathyroid small droplets or granules of a fatty nature, which increase in number with age, and are apparently independent of the general nutritive condition of the body.

Pepere ('06) in his extensive work on the parathyroid gland mentions briefly (p. 265) some observations upon the effects of inanition on the parathyroid in dogs starved 9 to 27 days, and in two human cases of death from inanition following oesophageal obstruction. The parathyroid is found relatively less affected than any other viscera. In the dog there is atrophy of the parenchyma, especially of the cytoplasm, which becomes greatly reduced in amount, with vacuolization and loss of the characteristic granulation. The nucleus is hyperchromatic. In the human parathyroid, the effects of inanition are similar, including: "*rimpicciolimento dei corpi cellulari, diminuite le granulazioni protoplasmatiche, scarsissimi gli elementi cromofili, estese degenerazioni idropiche e vacuolari delle cellule, assente la colloide.*" Thus Pepere's observations upon the human and canine parathyroids are in general agreement with my findings in the rat, excepting that he does not mention the occurrence of (advanced) pycnosis or karyolysis.

According to Thompson ('06) the parathyroids in man cannot be said to have a distinct pathology, although various degenerations and other pathologic conditions have been described. He cites the work of Peterson and of Benjamins, in which parenchymatous atrophy, hydropic degeneration, etc., are shown to occur frequently in the human parathyroid.

Following the description by Welsh ('98) there are generally described in the parathyroid of man (and some of the lower ani-

mals) two types of cells: (1) 'principal' cells described by Welsh as having in man "a relatively small and clear protoplasmic body, and a relatively large and clear nucleus." These cells constitute the chief part of the gland, and are constantly present. (2) The 'oxyphile' cells usually have relatively more cytoplasm, finely granular and sometimes finely vacuolized. The nucleus is both relatively and absolutely smaller than in the 'principal' cells, its outline more circular and chromatin more dense, "so that no nuclear network can be detected and the entire nuclear mass appears uniformly and deeply stained." These 'oxyphile' cells are found by Welsh in a very large proportion of cases, though not all, and never so abundantly as the 'principal' cells. They never form a large proportion of the gland, and may occur as small masses (often just beneath the capsule) or as scattered cells throughout the gland.

The presence of these 'oxyphile' cells has been confirmed in man and a part (not all) of the lower animals. They are generally considered to represent cells in functional activity. Against this view, however, is the fact that they are quite variable in appearance, and frequently absent altogether (sometimes in man; constantly in some of the lower animals, and especially in the young).

Although I have made no special study of the parathyroid in any other form, the conditions found in the rat lead me to suggest the possibility that these 'oxyphile' cells may represent merely atrophic types, with no functional significance. It will be noted that (although the cytoplasm is relatively abundant) the nucleus of these 'oxyphile' cells as described by Welsh and others corresponds closely with the pycnotic condition characteristic of atrophic, degenerated cells, such as are described above in the normal parathyroid and thyroid glands, and are increased in number during inanition. While the evidence at hand is insufficient for a final conclusion, it seems to me that this interpretation is a possibility which should be kept in mind during further investigation of the matter.

SUMMARY

The more important results of the present investigation may be summarized as follows.

In young rats held at maintenance for several weeks (and hence in a condition of chronic inanition), the histological changes in the thyroid are varied. The follicular epithelium is atrophied, with reduction in height. The nuclei are rarely hypochromatic (various stage of karyolysis), but hyperchromatosis is more typical, the nuclei usually presenting some stage of pycnosis. In the earlier stages the nucleus may be nearly normal in size and structure, excepting a pale, homogeneous coloration of the nuclear background. In more advanced stages, the nucleus diminishes in size, with deepened coloration, forming a dense, deeply-staining, homogeneous mass (typical pycnosis). In extreme cases the nucleus becomes fragmented (karyorrhexis). Neither mitosis nor amitosis is found.

The cytoplasm is usually reduced in amount considerably more than the nucleus. The cytoplasm may show no marked change in structure (simple atrophy), but usually becomes rarefied, with a marked vacuolization ('hydropic degeneration') and loss of the normal granulation. This is especially marked in the few cells where the cytoplasm has lost but little in volume. In some cases the cytoplasm may become homogeneous ('colloid' type) and in advanced stages may disintegrate, forming irregular, deeply-staining (eosinophile) masses of varied appearance.

The intrafollicular colloid may show no abnormal changes. Advanced stages of degeneration in the follicular epithelium, however, are accompanied by dissolution and disintegration of the colloid. The colloid is often replaced by desquamated epithelial cells in various stages of degeneration, and the entire follicle may collapse into an irregular mass.

The interfollicular connective tissue (stroma) usually shows no very marked change in structure, but is often increased in volume by an infiltration of ground substance, giving a somewhat edemic appearance. On this account, the whole thyroid

gland may show but little loss in absolute weight, although there has been a marked atrophy of the parenchyma.

In the adult rats subjected to acute and chronic inanition, the changes in the structure of the thyroid gland are likewise varied, but in general similar to those found in the younger rats. The interpretation of the changes in the older rats is more difficult, on account of the frequent occurrence in the normal (control) rats of degenerative changes somewhat similar to those found in advanced stages of inanition.

These changes, involving desquamation and degeneration of the follicular epithelium, have frequently been observed in the thyroids of rats both normal and under various abnormal conditions. They also occur as pathological changes in various other glands. It is suggested that the similarity of these cell-changes may possibly be due to cell-inanition as a common underlying factor.

The parathyroid glands appear to be relatively larger in the female. They apparently belong to that group of organs in which growth persists in young rats, even when held at maintenance (constant body weight) by underfeeding. In adult rats during acute and chronic inanition, the reduction in the size of the parathyroids is nearly proportional to that of the body as a whole.

In histological structure, the parathyroid gland is relatively more resistant than the thyroid to inanition. The changes in the structure of the epithelial cells are somewhat similar to those described for the thyroid, though in general less marked. In many of the cells there is apparently no decrease in the average size, but some (especially those degenerated) show marked shrinkage. The nuclei may remain nearly normal in size and structure, though usually exhibiting various stages of (rarely) karyolysis or (more frequently) karyopycnosis. No cell-division is found. The cytoplasm may be either somewhat reduced in amount, sometimes deeply-staining ('oxyphile'), or may remain nearly normal in volume, with marked vacuolization ('hydropic degeneration'). The stroma may remain normal in

amount, but is occasionally increased in volume by infiltration of ground substance.

Variations also occur normally in the structure of the parathyroid (though less marked than in the thyroid), so that here likewise caution must be observed in drawing conclusions as to effects of experiments. The 'oxyphile' type of cell does not appear to be a normal constituent of the parathyroid gland in the rat. However, some of the degenerated cells resemble in many respects the usual description of this 'oxyphile' type, which therefore may possibly represent an atrophic rather than a functional condition.

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STUDIES ON THE MAMMARY GLAND

I. THE GROWTH AND DISTRIBUTION OF THE MILK-DUCTS AND THE DEVELOPMENT OF THE NIPPLE IN THE ALBINO RAT FROM BIRTH TO TEN WEEKS OF AGE

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SIX TEXT FIGURES AND FOUR PLATES

The mammary gland undergoes many important changes from birth to old age. Numerous details in the normal structure of the gland at various stages of its history are still imperfectly known. Because of the lack of anatomical knowledge, pathologists are often unable to determine whether a certain condition of the mammary gland is due to a physiological or a pathological change. Moreover, a knowledge of the normal course of development is necessary as a basis for various lines of experimental work upon the mammary gland. In view of these facts further investigation of the developmental changes in the mammary gland during its life history seems desirable. Hence a series of studies upon this subject has been undertaken. The present paper, which is the first of the series, deals only with the growth and gross relations of the ducts, and the gross development of the nipples from the first day to ten weeks after birth. In later papers, the prenatal condition as well as various changes involved during pregnancy, lactation, and involution will be considered.

MATERIAL AND TECHNIQUE

The present study is confined to the mammary gland of the albino rat (*Mus norvegicus albinus*). This form is easily reared in the laboratory and is thus available at all times. Its life cycle is short, so that any desired stage in the history of the mammary

gland can be obtained in a comparatively short time. The various developmental stages are therefore easily obtained and controlled.

In the present study three methods were used. (1) Microscopic sections were studied; (2) wax reconstructions (of the newborn) were made according to Born's method; (3) the ducts were studied from cleared preparations. These preparations were made according to the method employed by Lane-Claypon and Starling ('06). The skin of the entire ventral part of the body was removed, spread out on a sheet of cork, and fixed in a mercuric chloride-formalin solution (10 per cent formalin in a saturated aqueous solution of mercuric chloride). The corium and tela subcutanea containing the gland were then removed in a single sheet. In the older specimens it was usually necessary to dissolve out the fat with alcohol and ether before staining. The preparations were then placed in a very dilute solution of alum-hematoxylin or carmalum until they were sufficiently stained. When necessary, the excess stain was washed out with acid-alcohol. After dehydration, beechwood creosote and cedar oil were used as clearing agents, the specimens being first placed in creosote for a few hours and then transferred to cedar oil. After being thoroughly cleared they were mounted in damar on glass slides.

Frank and Unger ('11) state that Starling's technique of staining and clearing the breasts, as in the rabbit, could not be employed in the rat. No explanation is given as to why Starling's method could not be employed. It is true that this method is rendered quite difficult owing to the development of the panniculus carnosus muscle in the thoracic region. Being very closely related to the milk-ducts, it is almost impossible to dissect this muscle off without destroying some of the mammary gland. However, good cleared preparations can be studied to advantage even when the muscle is intact. The fact that the panniculus carnosus muscle is lacking in the abdominal and inguinal regions makes the study of the glands in these regions comparatively easy. Fat is quite easily removed in cleared preparations, hence the considerable quantities of it deposited in the

region of the abdominal and inguinal glands do not add many difficulties in the technique.

Sections for microscopic study were prepared by fixation in Zenker's fluid, and embedding in paraffin. The sections were cut 10 micra thick, mounted serially and stained with Mallory's connective tissue stain or with alum-hematoxylin.

The albino rats used were in good health. After weaning, at the age of three weeks, they were fed upon whole wheat (Graham) bread, soaked in whole milk. They were in general of average weight or above, as indicated by the following gross body weights of the rats from which the specimens represented in the figures were obtained: newborn, about 4.5 grams; 1 week, 8.5 grams; 2 weeks, 15.5 grams; 3 weeks, 30 grams; 4 weeks, 53 grams; 5 weeks, 54 grams; 7 weeks, 75 grams; 9 weeks, 114.5 grams.

OBSERVATIONS

1. General arrangement of glands and ducts

As shown in figure 1, the mammary glands of the albino rat may be grouped according to the regions which they occupy. The three most cephalic pairs of glands lie in the thoracic region, hence are designated thoracic mammary glands. Passing caudad, the next pair lies in the abdominal region at about the level of the umbilicus. Henneberg ('00) speaks of this pair as the abdominal mammary glands. The two remaining pairs, known as the inguinal mammary glands, lie in the inguinal region.

Each of the first pair of thoracic glands lies slightly cephalad to, or at the level of, the fore limb. Each adult nipple is located about 10 to 12 mm. from the mid-line. In the case of the second pair of thoracic gland as a nipple is found immediately behind each forelimb. Here the distance from the nipple to the mid-line is somewhat greater, being about 15 to 16 mm. The distance between this and the first pair of thoracic glands is normally about 30 mm. The last pair of thoracic glands is very closely associated with the second pair, the nipples being only about 12 to 15 mm. apart. The distance from each nipple to

the mid-line is approximately 35 mm. The abdominal pair of glands stands somewhat isolated from the others, lying about 50 mm. caudad to the last thoracic and 25 mm. cephalad to the first inguinal. As stated above, these glands lie near the level of the umbilicus. Their distance from the mid-line is about the same as, or slightly greater than, that of the last thoracic glands. Each nipple of the first pairs of the inguinal glands lies in the



Fig. 1 Ventral aspect of adult female rat during lactation, to show location and arrangement of the nipples (from Henneberg '00).

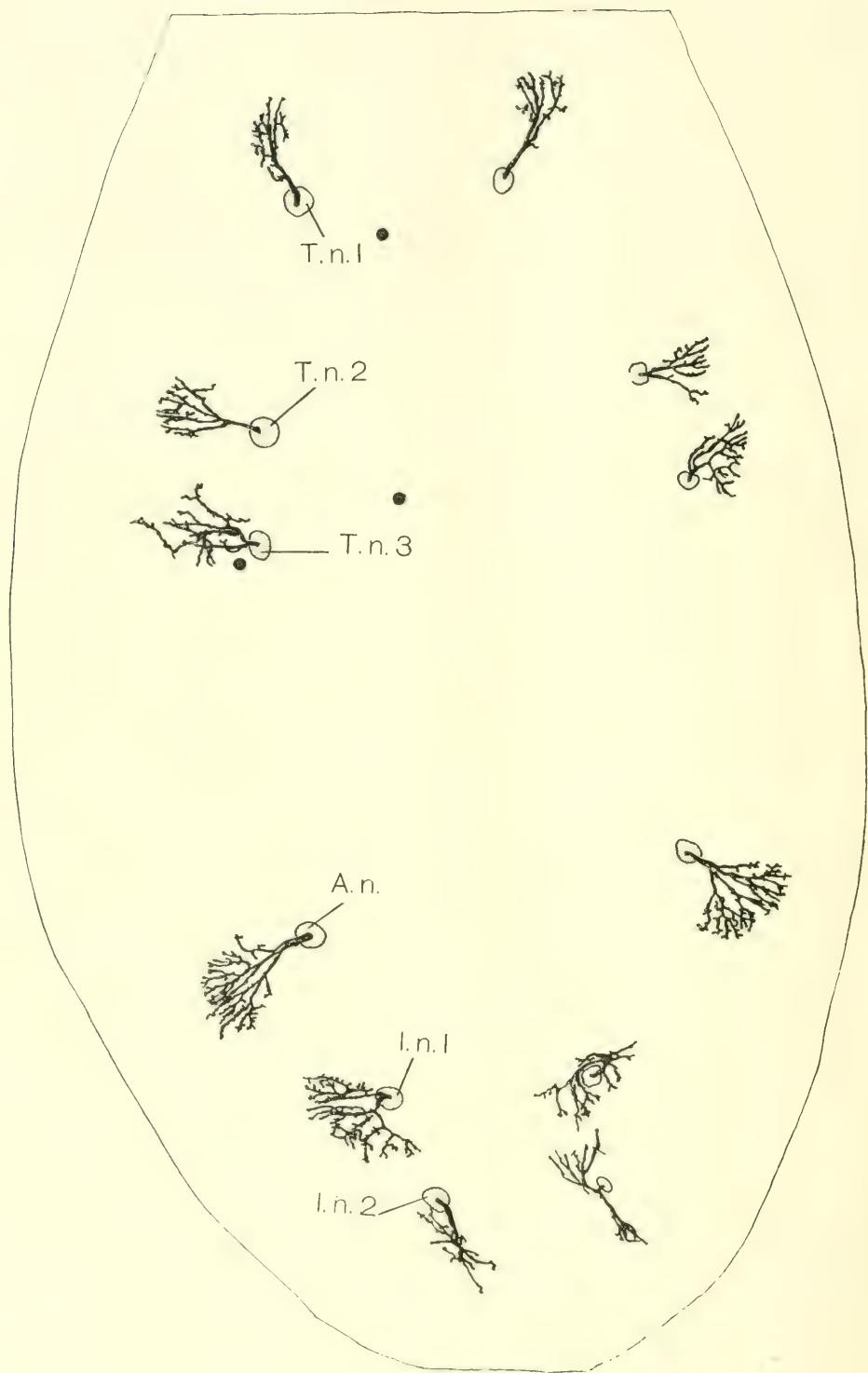
inguinal region immediately medial to the thigh. The distance from the nipple to the mid-line is approximately 12.5 mm. The nipples of the second pair of inguinal glands lie in the most caudal part of the inguinal region. They are located latero-cephalad to the urethral orifice. Like the first thoracic glands, the nipples of the last inguinal glands approach the mid-line, the distance being only about 10 to 12 mm. It should be observed that in passing caudad from the first thoracic glands the distance from the nipples to the mid-line gradually increases until the last

thoracic pair is reached, remains about the same for the abdominal pair, then decreases to the last inguinal pair.

From figure 1 and the above measurements it will be observed that the abdominal pair of glands is more closely associated with the inguinal than with the thoracic glands. This arrangement points toward a localization of the glands in only two regions. So far as the distribution of the mammary glands is concerned, the rat may be regarded as occupying a position between those forms which possess a continuous row of mammary glands on each side from the thoracic through the inguinal regions and those in which the mammary gland is confined to a single region.

The average number of mammary glands in the albino rat is 12 (6 pairs), but Henneberg ('00) and Frank and Unger ('11) have called attention to the fact that the number varies between 10 and 14. Henneberg examined 28 embryos from the age of 14 days and 20 hours to 15 days and found in 5 cases that supernumerary mammary hillocks appeared. All were in the pectoral region. The accessory hillocks, which in all cases appeared smaller than the normal, were located in 4 cases between the second and third normal hillock, and in one case caudal to the third. In the adult animals, however, he found that hypermastia rarely occurred, as only one case was reported from 150 observations. In this one case the individual possessed a pair of apparently functional glands just caudad to the last pair of thoracic glands. In the 150 adult rats only one case of hypomastia was found. In this case the individual lacked the first pair of inguinal glands.

In the present study, the number of glands present was noted in all animals available. From observations made on 100 individuals ranging in age from 10 days to adults, 80 were found to possess the normal number of mammary glands. Only one supernumerary gland was observed. It was located just caudad to the third thoracic gland on the left side, the right side presenting the normal number. In 12 cases the second thoracic gland was apparently lacking on the right side only, while in 7 other cases the second thoracic gland was lacking on both sides.



A very good time to make these observations on the albino rat is about the tenth to the fourteenth day of life. As pointed out by Jackson ('12), the mammary glands (nipples) are very conspicuous at this time. After the first two weeks have passed, it is very difficult to make accurate observations as the glands are well covered with dense hair. During pregnancy and the period of lactation the glands again become very conspicuous.

Just medial to the apex of each nipple is a single opening which leads into one large duct. This duct after reaching the tela subcutanea turns almost at right angles and courses through this layer parallel to the surface. Instead of receiving a large number of tributaries from all directions, this single duct at first receives only a small number of tributaries usually from a single direction. Figure 2, drawn from a cleared preparation of the integument of a rat two weeks old, will serve to show the general direction taken by the ducts of each gland. Here it may be observed that the main duct of the first thoracic gland extends cephalad, then breaks up into numerous branches. No ducts are seen to pass out in any other direction from the nipple. The general direction of the ducts of the second thoracic gland is somewhat different as they pass almost directly laterad from the nipple. In a number of cases, however, these ducts were found to extend somewhat latero-cephalad. A larger number of branches lead from the third duct than from either of the other thoracic nipples. The ducts of the third take the same direction as do those of the second thoracic gland. The abdominal gland, whose duct shows a greater amount of branching than any of the others, sends its branches in a caudo-lateral direction. The main duct of the first inguinal gland, after passing a short distance caudo-laterad, breaks up into ramification some branches of which take a cephalic while others take a caudal direction. The duct of the last or second inguinal gland usually sends all of its branches directly caudad. In some specimens, however, a few branches

Fig. 2 Drawn from a cleared preparation of a two weeks' albino rat (internal view) to show the general arrangement of the nipples and the branching of the mammary ducts. $\times 4$. *T.n.1*, *T.n.2*, *T.n.3*; 1st, 2d and 3d thoracic nipples; *A.n.1*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples.

pass cephalad. It is evident from the drawing of the surface of the abdomen (fig. 1) that the nipples are arranged on each side so as to form somewhat of an arch. It should be noticed in figure 2 that the arch effect is also carried out by the direction and distribution of the ducts.

The milk-ducts of late human and rabbit fetuses were divided by Rein ('82) into several subdivisions as follows: (1) the inflated terminal branches of the 1st, 2d, and 3d orders which together form the outline of the secretory part of the gland; (2) that part extending from these ramifications to the mammillary zone which represents the future lactiferous sinus; (3) the intra-mammillary portion which constitutes the proper excretory duct; (4) the infundibuliform part which passes through the epidermis. Brouha ('05) was unable to adopt the subdivisions proposed by Rein, because neither at birth nor even in the course of the first month of postnatal life was he able to distinguish any part which was differentiated into a lactiferous sinus. Owing to this fact Brouha suggested and followed a somewhat different subdivision. He considers each milk-duct composed of three segments; (1) the intra-epidermal infundibuliform segment; (2) the excretory segment which extends from the preceding to the place of the first bifurcation; (3) the secretory segment, which is composed of the succeeding branches. This subdivision Brouha bases upon the later histological characters of the different portions of the lactiferous arborization.

A somewhat different classification of the ducts is used in this paper. That part of the duct passing through the epidermis is called the intra-epidermal portion of the primary duct. That part extending from the epidermis to the first division is designated the primary duct. The ducts resulting from the divisions of the primary ducts are spoken of as the secondary ducts. The secondary ducts divide into the tertiary ducts. The collateral ducts are those given off from the sides of the main ducts. All those ducts which end blindly are called terminal ducts.

When the various pairs of glands are examined it will be observed that for certain features a single general description can be applied to the ducts of all. However, each varies more or

less from a general type; and it thus becomes necessary in some places to make the description somewhat detailed. This description is given later under "Growth of the ducts."

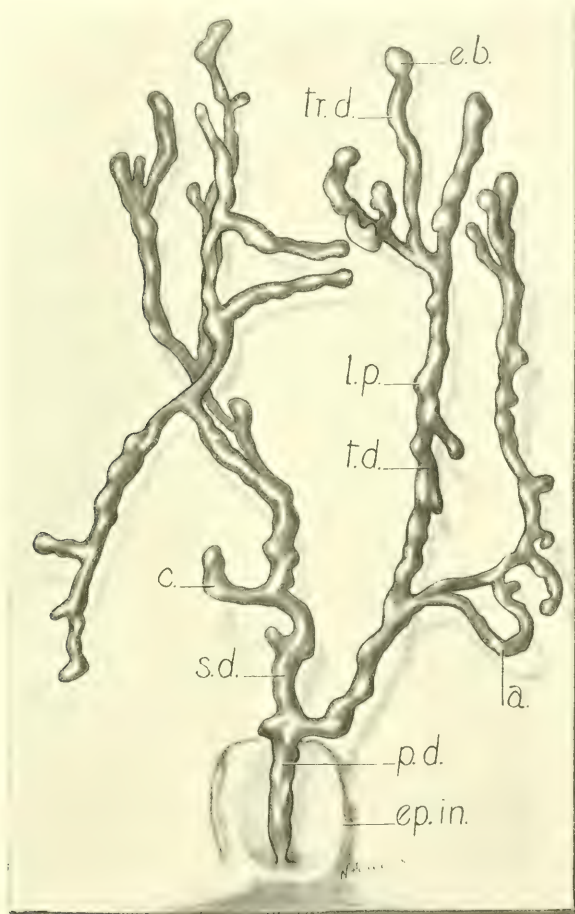


Fig. 3 Internal view of a wax model reconstructed from the left second thoracic gland of a newborn albino rat. $\times 40$. *a*, anastomosis; *c*, collateral duct; *ep.in.*, epithelial ingrowth of nipple; *e.b.*, end-bud; *l.p.*, lateral process; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct.

In figures 3 to 6 (from wax reconstructions of the newborn) the intra-epidermal portion of the primary duct is not visible,

but the primary duct is seen to emerge from the inner surface of the epidermal layer of the skin, pass deeply into the tela subcutanea and suddenly turn at right angles after which it lies paral-

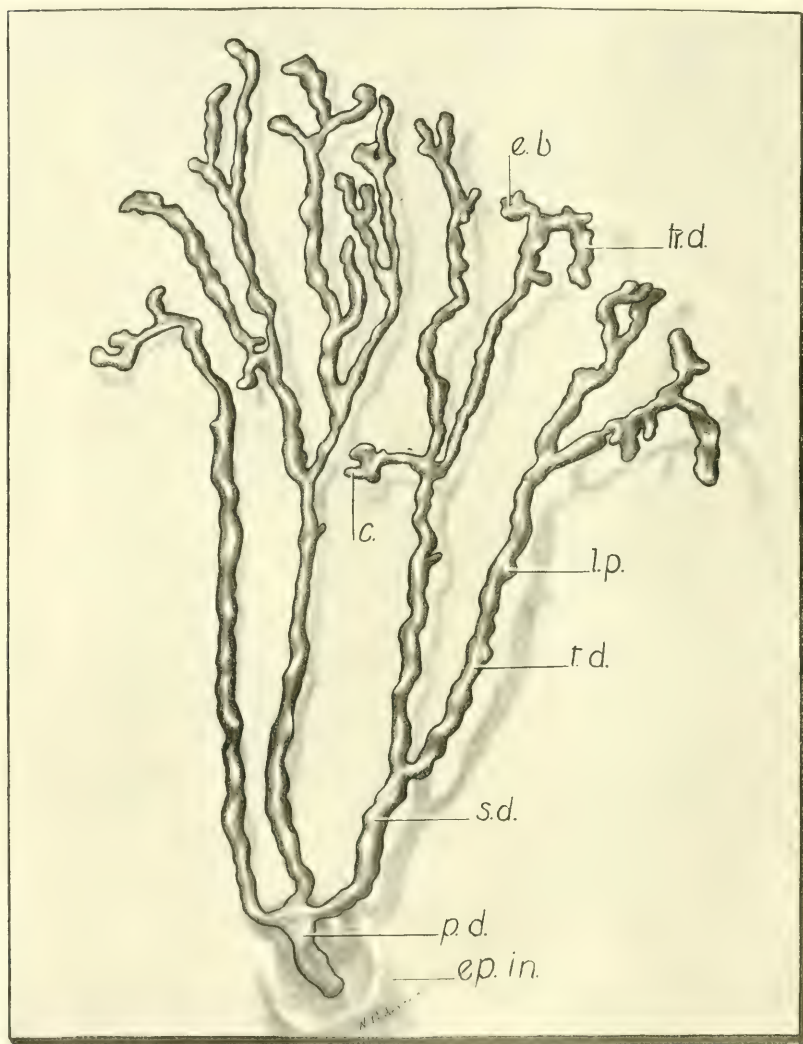


Fig. 4 Internal view of a wax model reconstructed from the right third thoracic gland of a newborn albino rat. $\times 40$. (For lettering, see fig. 3.)

lel to the surface of the skin. In most cases observed the primary duct extends only a short distance until it divides into two branches (secondary ducts) nearly equal in size.

The extent of the primary duct varies considerably. For instance, in the first thoracic and the last inguinal glands the pri-



Fig. 5 Internal view of a wax model reconstructed from the left first inguinal gland of a newborn albino rat. $\times 40$. (For lettering, see fig. 3.)

mary ducts present a rather extensive course before dividing, while in the remaining glands they divide almost immediately after making a sharp turn in the tela subcutanea. In figure 4 (last thoracic gland) the primary duct is seen to divide into three branches. This is an exception to the general rule that the primary duct divides into two branches.

As compared with the primary duct, the secondary ducts present a rather extensive course, after which they break up each into two or more branches (tertiary ducts). It will be noticed that at birth (figs. 3 to 6) the terminal branches of each tertiary duct vary from one to three in number. On the end of most terminal branches is a small bud-like enlargement. These enlargements were described as true alveoli by earlier investigators, but this was found later to be incorrect. Billroth (according to Berka '11) doubts whether completely formed end-vesicles occur in young human virgins. While he called the terminal enlargements 'real end-vesicles,' yet he adds that they later develop into 'true end-vesicles' and further multiply during pregnancy. Berka ('11) states that true alveoli do not occur in young (human) virgins. Similarly the terminal enlargements found on the milk-ducts of young rats are not true alveoli, but are only enlarged growing processes corresponding to the end-buds found in other developing glands. The microscopic structure of these enlargements and the development of true alveoli will be discussed in a later paper dealing with the histology of the mammary gland.

The question often arises as to whether the ducts of glands branch dichotomously or otherwise. From the various figures it will be seen that the more proximal parts of the terminal segments usually follow the dichotomous method, but the distal portions, as stated above, may terminate as a single duct or divide into two or three branches. In the last thoracic gland (fig. 4) the secondary branches approach true dichotomous division.

Anastomoses occur between ducts, but they are not very frequent in the newborn rat. In the reconstructions made from glands at birth, only two distinct anastomoses occur (fig. 3). However, others have been observed in cleared preparation at the same stage.

It will be noted that along the secondary and tertiary ducts numerous lateral buds occur (figs. 3 to 6). Many more of them are present on the distal than on the proximal ducts. Such buds later form collateral branches destined to develop into ducts similar to those already present. This point will be more clearly brought out in the older stages.

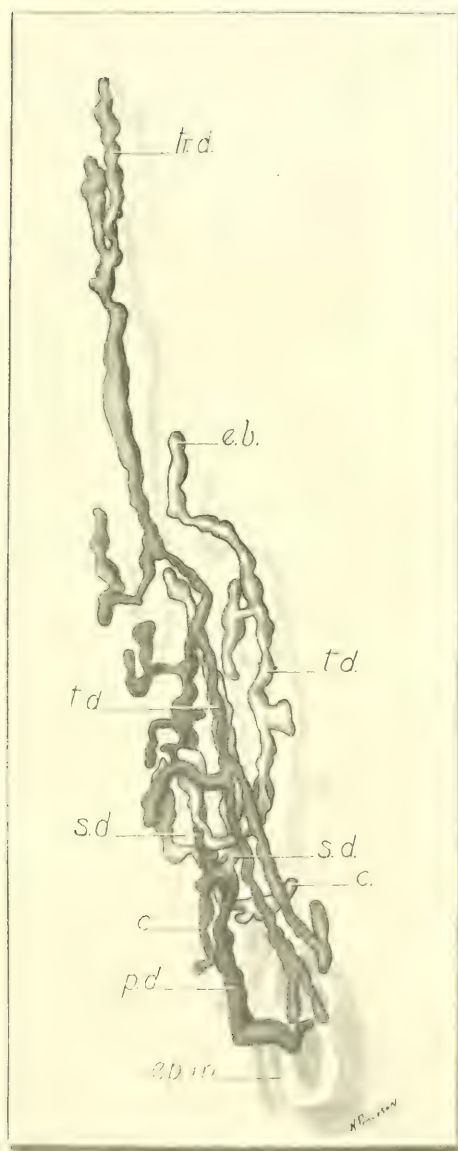


Fig. 6 Internal view of a wax model reconstructed from the left second inguinal gland of a newborn albino rat. $\times 40$. (For lettering, see fig. 3.)

A point which has been discussed at some length recently and one which has proved to be of considerable importance in experimental work is that of the variation in the relative size and development of the various glands in the same individual, and of glands from different individuals of the same age. Lane-Claypon and Starling ('06) in working on the growth and activity of the mammary gland concluded that breast hyperplasia of pregnancy is caused by chemical substances formed in the embryo. Such substances passing through the placenta into the maternal blood-stream cause growth of the mammary gland. To decide definitely as to just what tissues cause this growth Lane-Claypon and Starling injected extracts of placenta, placenta and uterus, ovaries, fetus, fetus together with the placenta and membranes, and mucous membrane of the uterus into virgin rabbits. Some of the extracts when injected caused very little apparent change in the size of the mammary gland of virgins, while others (fetus extract, for example) seemed to cause a marked development of the glands.

Frank and Unger ('11) in repeating certain of Lane-Claypon and Starling's experiments obtained different results, and furthermore found that their own series of experiments did not show uniform results. Thus they concluded that some disturbing factor remained to be accounted for, so they decided to study more carefully the anatomy and the physiology of the normal mammary glands of the rabbit. For such study they selected a number of apparently virgin adult female rabbits and under the necessary precautions removed a mammary gland from each. At various intervals of time other mammary glands from the same animal were removed and studied. From these experiments Frank and Unger were able to demonstrate in virgin rabbits changes which were indistinguishable from those seen at the end of the first third of pregnancy. Thus some physiological factor must be involved. Frank and Unger found a partial explanation for this condition in an article by Bouin and Ancel ('09) who describe variation in the size and appearance of the rabbit's mammary gland corresponding to the development of the corpus luteum. A little later O'Donoghue ('12) showed that

there is a decided change in the structure and size of the mammary glands of *Dasyurus viverrinus* when ovulation is not succeeded by pregnancy.

A comparison of the individual glands of the rat at birth and at two weeks (figs. 2 to 6) will show that there is considerable difference in the size and development of the various glands in the same rat, sometimes even in the same pair of glands (fig. 2). It has also been observed that corresponding glands from different rats of the same age and approximately equal weights show considerable variation in size and complexity of structure. The differences in size and development observed by me in the rat are not so marked as those described by Frank and Unger, Bouin and Ancel, and O'Donoghue. Yet they are worthy of mention and are certainly sufficient to prove that the normal structure and variability under different conditions of any part of the animal body should be thoroughly investigated before conclusions are drawn from experimental work. It is quite possible that such knowledge of the mammary gland of the rabbit would have changed decidedly the conclusions of Lane-Claypon and Starling.

2. Growth of the ducts

In the newborn rat, models were reconstructed showing one gland of each of the six pairs (figs. 3 to 6). At two weeks, all the glands are represented in figure 2, to show the general topography of the ducts. At the other stages (1 week, 2, 3, 4, 5, 7 and 9 weeks, figs. 7 to 13) it is found unnecessary to reproduce all the glands, so only the abdominal and inguinal glands of the left side are shown. A general description of all glands at each stage will be given, however.

Newborn. The figures drawn from wax reconstructions of the glands at birth (figs. 3 to 6) show that with few exceptions the ducts of each gland in these stages all lie approximately in the same plane, parallel to the surface of the skin. It was noticed that in regions where there are no obstructions the ducts spread very freely and cover a considerable area. The unobstructed ducts almost invariably lie in a single plane at this stage of

growth. The last thoracic and the abdominal glands (fig. 4) are good examples. Here, as is readily seen, the integument is free from appendages or anything that would tend to limit the uniform spreading of the ducts. It was also noticed that in regions where there are obstructions the branching of the ducts is more irregular and that the ducts arrange themselves so as to lie in more than one plane. The best example of this condition is seen in the last inguinal mammary gland (fig. 6). Here as previously stated the nipple lies in the caudal part of the inguinal region. To the lateral side of this gland the area available for ramification of ducts is obstructed by the hind-limb while to the medial side the external urinary and genital organs limit the area. This leaves only a very narrow region free for the distribution of ducts. Consequently, instead of spreading freely and occupying a single plane, the ducts branch so as to lie in three or four planes, each of which is parallel to the surface. Small areas where the growth of the mammary gland is obstructed by lymphatic glands are shown in several of the figures of later stages. Thus the course, branching, and spreading of the ducts depend largely upon the available space.

The reconstructions show that the ducts of the abdominal and inguinal glands lie much deeper from the surface than those of the thoracic glands. This is probably due to the absence of the panniculus carnosus muscle which may to some extent prevent the ducts from passing deeply in the thoracic region. Also considerable fat is present in the abdominal and inguinal region, which is apparently a very favorable substance for the ramification of ducts.

First week. At the end of the first week the ducts are not much different from those at birth except that they are slightly more branched. The ducts of the first thoracic glands give off a few branches which take a caudal direction; all the other branches of this pair of glands pass cephalad. The second pair of glands sends the ducts in a latero-cephalic direction, but many collaterals are given off some of which take a cephalic while others take a caudal direction. The third thoracic glands send their ducts in the same general direction as the second. The

greater number of the collateral ducts of this gland pass so far cephalad that only a comparatively small space exists between them and the caudal collaterals of the second thoracic gland. The caudal collaterals are few in number, yet quite long.

The ducts of the abdominal glands (fig. 7), which are most branched of all, send their branches in a latero-caudal direction. Many collaterals are given off, more of which take a caudal than a cephalic direction. In the case of the first inguinal gland, the number of collateral branches taking the cephalic direction is about equal to the number taking the caudal direction. The ducts of the last inguinal glands send the majority of their branches directly caudad; however, a few branches may be seen passing cephalad toward the first inguinal gland. Terminal end-buds are very prominent on all the glands at this stage. Also the small lateral buds are numerous but they are largely confined to the more distal ducts.

Two weeks. Figure 2 represents all of the mammary glands of a rat at the end of the second week of life. Figures 2 and 8, together with the description given in an earlier part of this paper, render further description unnecessary.

Three weeks. The three weeks' specimen from which figure 9 was drawn shows less branching than the one-week stage, but the ducts are greater in diameter. This specimen greatly emphasizes the marked variation in the development of the glands in different individuals, since in this instance the glands of an individual one week old show greater development than those of another individual three weeks old. Also, as pointed out above, the glands of one side may show more advanced stages of development than corresponding glands of the opposite side.

Four weeks. At the end of four weeks the first pair of mammary glands does not show a marked increase in development over the two-weeks stage. One very noticeable difference is that the proximal parts of the secondary ducts bear a large number of lateral buds similar to those appearing on the distal parts of the same segment of the earlier stages. Such processes are much less numerous on the corresponding parts of the second and third thoracic glands. The second glands in some of the speci-

mens show a very slight increase in development over the two-weeks and three-weeks stages. The ducts of the third thoracic glands show a much greater development than has been observed in any of the earlier stages. The ramifications are so numerous that some are seen to pass superficially while others take a deep course. This arrangement necessarily takes them out of the plane of the main ducts. Thus from this stage the ducts become so crowded that it is impossible for them all to occupy a single plane, as found at birth in all except the last inguinal glands. Large numbers of branches from the ducts in the third thoracic gland also take a cephalic course, which is so extensive on the right side that they very nearly come in contact with the ducts of the second thoracic gland. On the left side the interval between the ducts of the two glands is greater. Figure 10 shows that the abdominal and inguinal glands present much richer arborizations than corresponding glands of earlier stages. Some lateral buds are developed on the secondary ducts yet they are not so numerous as in the first thoracic gland of the same stage. The general course taken by the ducts is the same as that given in previous descriptions. A well defined interval exists between the ducts of the abdominal and the first inguinal glands. There is also a considerable space existing between the ducts of the first and second inguinal glands. However, the second inguinal has sent numerous ramifications in the direction of the first inguinal gland.

Five weeks. During the fifth week the ducts increase very rapidly in length. Also a great many new branches spring from the more distal ducts of the glands. The first thoracic gland is much more complicated than in any of the previous stages studied. Between the ducts of this and those of the second thoracic gland a wide interval still exists. The interval between the ducts of the second and those of the third thoracic glands has largely disappeared on both sides, there being a slight overlapping of a few of the ducts from each gland. In the specimens observed, however, there are no anastomoses present between the ducts of the two glands.

During this week the abdominal and inguinal glands (fig. 11) also undergo very rapid development. This is especially noteworthy in view of the fact that the body weight of the rat from which the gland at five weeks was drawn was practically the same as that of the rat used at four weeks (fig. 10). The ducts of the abdominal and first inguinal glands at five weeks (fig. 11) interlace very intricately. With the aid of the microscope (especially the binocular) one can be reasonably sure that many of the ducts simply overlap, there being no anastomoses. However, there are areas in which it is impossible to decide definitely as to whether true anastomoses occur. This holds true in all the later stages. Further investigations are necessary to determine this point concerning anastomosis. In this same week the ducts of the second inguinal glands on each side have grown cephalad to meet, and in some places even overlap, the ducts of the first inguinal. Here the overlapping is not very complicated and one can see distinctly that no anastomoses occur. The ducts of the second inguinal gland taking a caudal direction branch very profusely.

Six weeks. At the end of the sixth week the glands do not differ greatly from the five-weeks stage. Some of the glands, the abdominal for example, show greater development. Others, as the second inguinal, reveal no increase; in fact, in some specimens they are less developed than those of the five-weeks stage. The terminations of the ducts of the first and second inguinal glands are separated from each other by considerable space.

Seven weeks. In the seventh week stage, the caudally directed ducts of the first thoracic gland have made considerable advance toward the cephalically directed ducts of the second thoracic gland. However, they are still separated by a space of five to eight millimeters in width. As in some of the previously described stages the ducts of the second and third thoracic glands overlap. The branching of the ducts of these glands is somewhat more complicated at this stage. The abdominal and inguinal glands (fig. 12) present very complicated systems of ducts; also the overlapping between the ducts of the first and second inguinal glands is very marked at this time.

Eight weeks. In the eight-weeks specimens, the first and second thoracic glands have increased to such an extent that their ducts overlap. This overlapping may occur on one side only, there being an interval between the corresponding ducts of the opposite side. Concerning the other glands of this stage, very little need be said except that the ducts have increased in length and new branches have been added. It should be pointed out that at this stage the rat possesses only four distinct masses of mammary gland tissue. All of the thoracic glands of each side have their ducts so interlaced as to form one apparently solid mass of ducts. Also the abdominal and inguinal glands of each side are so matted together that no dividing line exists between them.

Nine and ten weeks. The nine and the ten-weeks stages (see fig. 13) show a tremendous increase in development over the previously described stages. The ducts have spread out to cover a much larger area. Not only is the overlapping of ducts more complicated but each gland has produced a large number of medial and lateral branches. For example, some of the medial ducts of the first thoracic glands of each side have grown so near to the mid-line that only a narrow space separates them. In the case of the first inguinal glands the medial ducts actually reach the mid-line, and a slight overlapping of the ducts of opposite sides occurs. The medial ducts of the second inguinal glands almost surround the vagina, the ducts from the opposite sides very nearly meeting in the mid-line both cephalad and caudad to the vagina. At this stage the proximal or secondary ducts also bear large numbers of short collateral ducts which have developed from the lateral buds mentioned in the earlier stages.

It is frequently stated that from birth to puberty the human milk-ducts undergo very little development, merely keeping pace with the general body-growth. At puberty an abrupt change like that affecting the entire organism is said to occur. So far as the rat is concerned, an examination of figures 2 and 7 to 13 will show that in the virgin, from birth to the age of ten weeks, there are apparently two periods when the increase in the mammary gland ducts is somewhat marked. The first period occurs

about the fourth and fifth weeks. Whether there are definite factors causing this first increase has not been determined. It is possible that it is due to individual variation. It does not appear to be due to any greater relative increase in the body weight at this period, and is of doubtful significance.

The second and more important period of increase in the mammary gland occurs about the ninth week. Donaldson ('15) states that the female rat arrives at the age of puberty about 60 to 70 days after birth, the gonads indicating sexual maturity at the age of two months or less. Jackson ('12) however, found pregnancy to occur in one case at the age of seven weeks. Lantz ('10) cites from Buckland a case where a white rat is said to have given birth to 11 young at the age of eight weeks (and which accordingly must have become pregnant at the age of five weeks). These are very exceptional cases, however.

Jackson ('12) states that the vaginal aperture does not appear until the middle or end of the second month. There is considerable individual variation on this point. The average taken from fifteen observations by me is 8.3 weeks. Therefore, the marked increase in the development of the mammary glands of the rat between the eighth and ninth weeks evidently corresponds closely with the age of puberty. Thus the second marked increase in the size of the mammary glands is readily accounted for.

3. Lumen of the ducts

The time of appearance and method of formation of the lumina of the milk-ducts in various animals have been described by various authors. In the albino rat at birth a small irregular slit-like lumen is present in the primary duct (fig. 14). This lumen when traced proximally disappears in the intra-epidermal portion of the primary duct, but when traced distally becomes continuous with the more regular rounded lumen of the secondary ducts. The tertiary and in fact all of the remaining ducts at birth possess lumina throughout their entire extent. The terminal buds also present distinct lumina to within 20 or 30 micra of their distal extremities. The lumen of each bud does not

appear greater in diameter than that of other parts of the ducts but the walls of the bud are usually considerably thickened. In some places the lumina of the ducts at birth are partially filled with substance which resembles colostrum.

At the end of the first week, the lumen is considerably larger and extends farther into the intra-epidermal segment. Considerable quantities of the substance mentioned above are present in the lumina of all segments of the ducts.

Figure 16 shows that the lumen opens on the surface of the nipple at two weeks. At this stage it is larger and more oval throughout the primary duct than in the previously described stages.

In other stages up to the tenth week no marked differences were observed except that the lumina gradually become larger as the ducts increase in size (fig. 17). No true alveoli were observed, yet the end buds in the later stages are changed somewhat in appearance so as to approach, or at least suggest, beginning alveoli. In all the stages more or less of the substance mentioned in the earlier stages was observed. This agrees with findings of Berka ('11), who describes particles of fat in the human ducts even in the later virgin stages. He thinks the source of this fat is the colostrum secretion of the newborn and that it lies in the ducts for years.

4. Growth of the nipple

Owing perhaps to the fact that the rat is born in a somewhat immature state, the nipples show only slight development at the time of birth. In the newborn the nipple can be recognized by the naked eye only with difficulty, the nipple areas appearing slightly lighter than the surrounding tissue. A section through the nipple area at this stage reveals the fact that it is only very slightly elevated above the surface of the skin (fig. 14). The epithelium of this area is considerably thickened and the figure reveals an epithelial ingrowth on each side of the thickened portion. Reconstructions show that these epithelial projections are continuous around the nipple area and that through them a

definite epithelial hood is formed, as shown in figures 3 to 6. Through this hood, which is filled with loose connective tissue, the primary duct passes on its way toward the surface.

Very little change takes place in the appearance of the nipple during the first week of postnatal life. From the surface the nipple areas appear slightly more elevated than at birth. Figure 15, drawn from a section cut very obliquely, shows that the thickened area of epithelium has become more extensive, also that the epithelial projections (ingrowth) are slightly longer.

During the second week the nipple develops very rapidly. It is no longer necessary to speak of it as the nipple area, since from its size and shape it now resembles a true nipple. Figure 16 shows the nipple at the age of two weeks to be a very prominent structure with the epithelial projections (ingrowth) correspondingly well developed. At this stage the primary duct actually pierces the surface. As previously stated, the nipples are very conspicuous externally toward the end of the second week of life, and from the tenth to the fourteenth days it was found easier to observe the glands externally than at any other time during the virgin state. During the third week, the nipples become hidden by the development of the hair coat.

Figure 16 also shows that at the age of two weeks a sulcus is beginning to appear between the nipple and the skin of the surrounding region. This sulcus is immediately superficial to the epithelial ingrowth mentioned above. Beyond the two-weeks stage (up to ten weeks) no changes have been observed to take place in the nipple, except that it slowly increases in size. This is shown in a nine-weeks stage (fig. 17) in which the structure is similar to that in figure 16, the chief difference being one of size.

SUMMARY

The results of the present investigation of the growth and gross relations of the ducts and the nipples of the albino rat from birth to the tenth week may be summarized as follows:

1. Observations made on 100 rats show the number of glands varies between 10 and 13, the normal number being 12 (6 pairs).

Only one supernumerary gland was observed. The second thoracic glands are those most often absent.

2. Only one primary duct is present in each gland. This duct after reaching the tela subcutanea turns at right angles and pursues a course parallel to the surface of the skin. The majority of the branches of ducts belonging to the first thoracic gland lie cephalad to the nipple, while those from the second and third thoracic glands lie latero-cephalad to the nipple. In case of the abdominal and first inguinal glands the greater number of the ducts lie latero-caudad to the nipples, while in the last inguinal the ducts lie caudad to the nipples. The dichotomous method of branching frequently occurs, especially in the proximal branches.

3. Reconstructions and cleared preparations show that anastomoses sometimes occur between the ducts of a single gland. It is uncertain whether anastomoses occur between the ducts of different glands.

4. End-buds are present on a large number of terminal ducts at all stages studied. No true alveoli were observed. Large numbers of lateral buds are present on the sides of all ducts distal to the primary duct during the earlier stages. Such lateral buds later develop into branches of the ducts.⁶

5. Considerable individual variation in the development of the glands was noticed. Not only do the corresponding glands of opposite sides differ in their degree of development, but also the glands of one individual may be better developed than those of another even several days older.

6. The characteristic distribution and ramification of the ducts apparently depend upon the space available for their growth.

7. The growth and branching of the ducts goes on at an unusually rapid rate about the ninth week, probably corresponding to the age of puberty.

8. A distinct lumen is present at birth in all the ducts distal to the intra-epidermal portion of the primary duct. At the end of the second week the lumen extends to the surface of the nipple.

9. The periphery of the nipple area is marked by a hoodlike epithelial ingrowth. The nipples make the most rapid growth during the second week, toward the end of which time they are very conspicuous.

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PLATE 1

EXPLANATION OF FIGURES

7 Drawn from a cleared preparation (internal view) of an albino rat one week old (weight 8.5 grams) to show distribution and relations of ducts of left abdominal gland (*A*); left first inguinal gland (*B*); and left second inguinal gland (*C*). $\times 5$. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct; *e.b.*, end-bud; *c*, collateral duct.

8 Same as figure 7, but from an albino rat two weeks old (weight 15.5 grams). $\times 5$.

9 Same as figure 7, but from an albino rat three weeks old (weight 30 grams). $\times 5$.

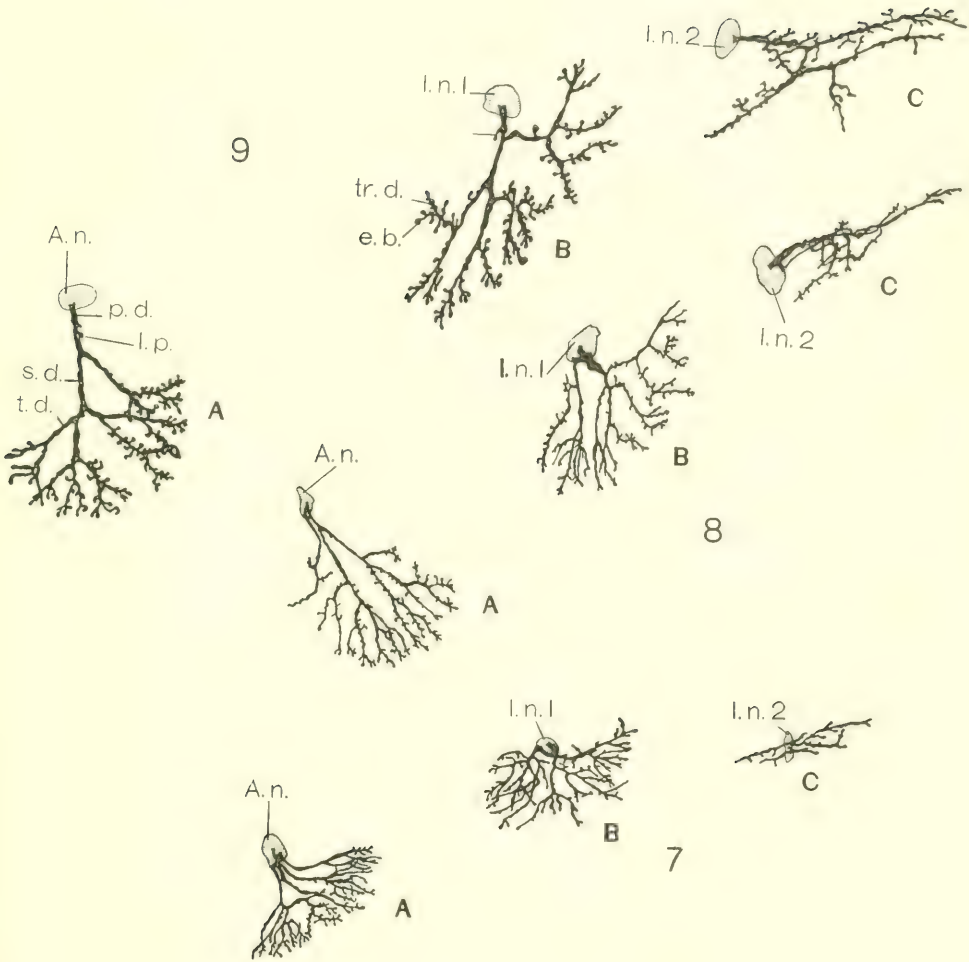


PLATE 2

EXPLANATION OF FIGURES

10 Drawn from a cleared preparation (internal view) of an albino rat four weeks old (weight 53 grams) to show distribution and relations of ducts of left abdominal gland (*A*); left first inguinal gland (*B*), and left second inguinal gland (*C*). $\times 5$. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct; *e.b.*, end-bud; *L*, lymph-node.

11 Same as figure 10, but from an albino rat five weeks old (weight 54 grams). $\times 5$.

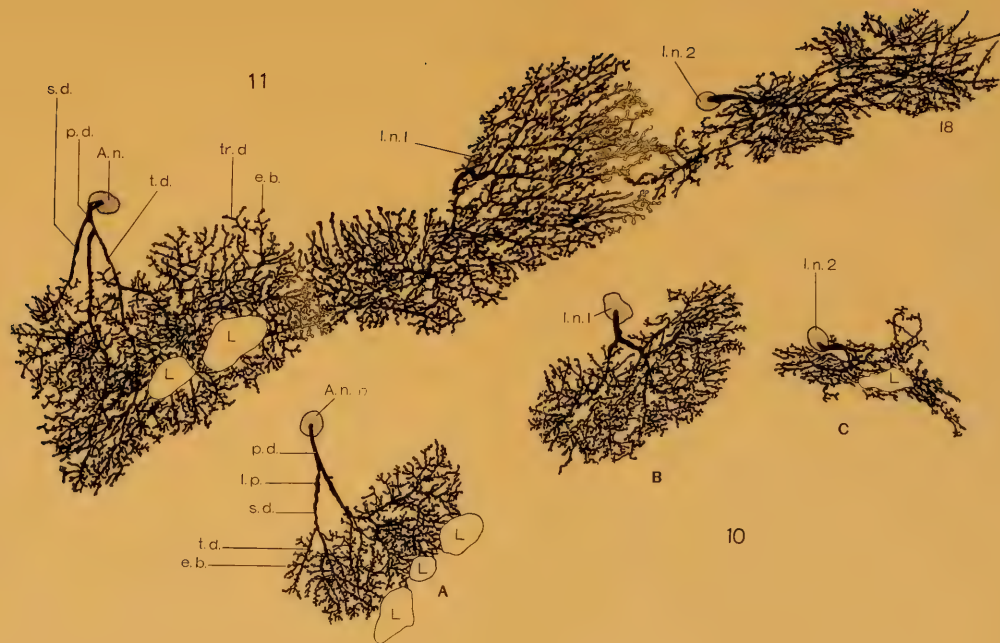


PLATE 3

EXPLANATION OF FIGURES

12 Drawn from a cleared preparation (internal view) of an albino rat seven weeks old (weight 75 grams) to show distribution and relations of ducts of left abdominal gland, first inguinal gland, and second inguinal gland. $\times 5$. *A.n.*, abdominal nipple; *In.1*, *In.2*, 1st and 2d inguinal nipples; *L*, lymph-node; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *c*, collateral duct; *tr.d.*, terminal duct; *e.b.*, end-bud.

13 Same as figure 12, but from an albino rat nine weeks old. (Weight 114.5 grams.) $\times 5$.

13



12

385

386

PLATE 4

EXPLANATION OF FIGURES

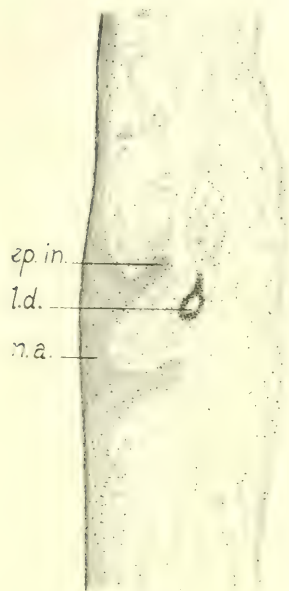
Lettering for figures 14 to 17 as follows: *ep.in.*, epithelial ingrowth of the nipple; *h.f.*, hair follicle; *l.d.*, lactiferous (primary) duct; *m.*, muscle; *n.a.*, nipple area; *s.*, sulcus at base of nipple.

14 Drawn from a section through the nipple area of the second inguinal gland of a newborn albino rat. $\times 67$.

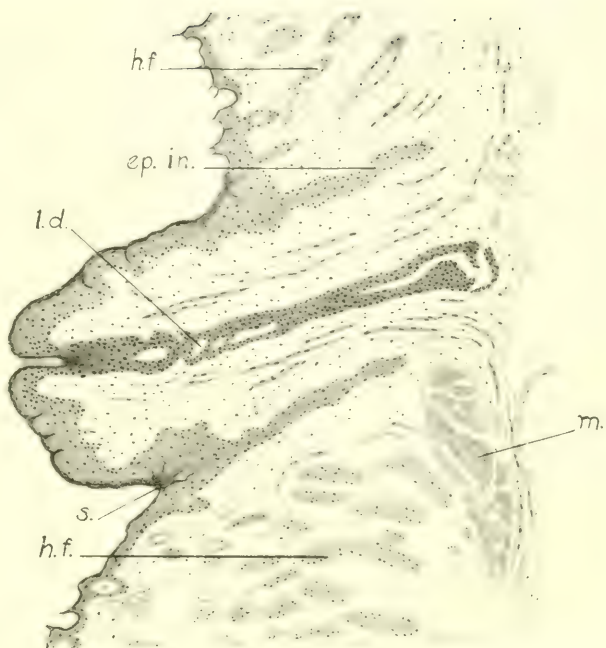
15 Drawn from a section through the nipple area of the second inguinal gland of an albino rat one week old. $\times 67$.

16 From a section through the nipple area of the second inguinal gland of an albino rat two weeks old. $\times 67$.

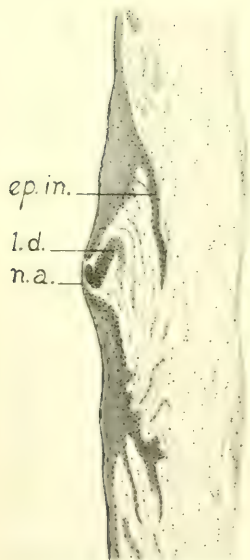
17 From a section through the nipple of the second inguinal gland of an albino rat nine weeks old. $\times 67$.



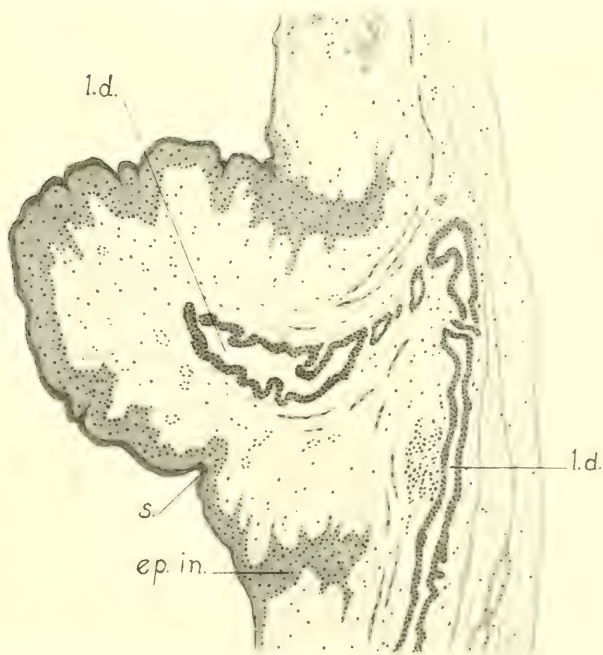
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16



15



17

THE RELATION OF CORONARY AND HEPATIC ARTERIES IN THE COMMON GANOIDS

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FOUR FIGURES

The regular occurrence of anterior and posterior coronary arteries in several of the Rajidae was definitely recognized by Hyrtl ('58) and T. J. Parker ('84). Subsequent investigations by numerous students of fish morphology have shown that while both sets of these arteries are characteristically present in skates, posterior coronaries do not occur in either the sharks or the teleosts. This fact has led to the prevailing idea that the posterior coronary artery is peculiar to the Batoidei. Such a vessel, however, has been found in the ganoid, *Polyodon*, by Allen ('07) and Danforth ('12).

In the latter paper the writer also pointed out the existence of anterior hepatic arteries arising in connection with the posterior coronaries. These anterior hepatic arteries were found to have a relation to the hepatic vein similar, in general, to the relation of posterior hepatics to the portal system. They supply the anterior half of the liver and usually part of the gall bladder, anastomosing here and elsewhere with the posterior hepatic arteries. That they may also occur in skates is indicated by a single observation on *Raja ocellata* where a typical anterior hepatic artery was found extending to the gall bladder.

The rather unexpected occurrence of posterior coronary arteries in *Polyodon* and the discovery of the anterior hepatics, has prompted the present investigation into the status of these vessels in other ganoids. For this study the crossopterygian *Polypterus* has not been available, but the laboratory has furnished abundant material representing at least one genus of each of the remaining four orders of recent ganoids. The following

species, one from each order, have been studied: *Scaphirhynchus platyrhynchus*, *Polyodon spathula*, *Lepidosteus osseus*, *Amia calva*. Specimens of all these fish have been kept in the aquarium to be killed and injected as needed. Some of them were operated upon and certain vessels tied several days or weeks before the fish was finally killed. About thirty specimens have been studied. A few of them were dissected without injection, but in most cases a colored starch mass was forced into the dorsal aorta as soon as the fish was killed. By this method all the vessels considered in the present paper are easily filled. The dissections have been supplemented by a study of serial sections of embryos and pieces of adult heart and liver.

The account of the conditions found in these four ganoids may be prefaced by a brief statement of the relations of hepatic and coronary arteries in lower forms. In this connection, it may be stated at once that the hepatic arteries described for sharks and skates (e.g. by Cavalié, '04) are the posterior hepatics. These are rather variable vessels, two of which usually arise from the coeliac artery and accompany the portal vein. In addition, other small arteries, from a similar source or from the oesophageal trunk, generally enter the liver. The number and arrangement of these arteries is far from constant even within a single species. In the present study, except for the few points mentioned below, I have found nothing about them calling for special comment.

A systematic interpretation of the relations of the coronary arteries in fishes was first proposed by T. J. Parker ('84, '86). According to his view, the whole region beneath the pharynx and in front of the pectoral girdle is supplied, typically, by a pair of vessels arising from the subclavian arteries. These vessels he designates as hypobranchials. In their course forward they give rise first to posterior (in skates) and then to anterior coronary branches, finally ending in anastomoses with the recurrent branches of the efferent branchial arteries. The posterior coronary arteries reach the heart by passing into the septum transversum, the anterior by following the aortic bulb backward. Later writers, among whom may be mentioned in particular G. H. Parker and K. Davis ('99) and Ferguson ('11),

have pointed out that the connection between the subclavian and hypobranchial arteries is generally slight or actually lacking. This is interpreted to mean that the two systems are essentially distinct with only secondary anastomoses. This being the case, the posterior coronary arteries of skates are derived from the subclavian artery, the anterior coronaries from the true hypobranchial system. Parker and Davis ('99) designated the branch of the subclavian from which the posterior coronary arises as the coracoid. They also differentiated clearly between median and lateral hypo-branchial arteries and recognized the existence in *Carcharias* and other forms of dorsal and ventral anterior coronaries.

It will be apparent from the foregoing that a discussion of the morphology of the coronary and hepatic arteries of ganoids will involve some consideration of the subclavian and hypobranchial arteries. The accompanying schematic drawings show the essential relations of these vessels in the fishes studied.

The principal branches of the subclavian artery are fairly constant. After reaching the level of the lateral line the main trunk gives rise above to a dorsal and below to a ventral superficial branch to the lateral body musculature. Following Pitzorno ('05), these may be designated as Rr. thoracico-dorsalis and thoracico-ventralis. Between these two vessels arise branches to the abductor and adductor muscles of the fin and a branch which runs forward beneath the heart to supply the Mm. coracoarcualis and pharyngoclaviculares. This is the coracoid artery of Parker and Davis (*l. c.*).

The hypobranchial system of vessels is somewhat rudimentary and highly variable. It does not lend itself readily to a general description.

The condition found in skates is most closely approached by *Scaphirhynchus*. This form will therefore be described first.

SCAPHIRHYNCHUS

In *Scaphirhynchus* anterior and posterior hepatic and posterior coronary arteries are present. I have not found anterior coronaries. The coeliaco-mesenteric artery passing down on the

right side of the oesophagus becomes embedded in the liver for a short distance near the pyloric end of the stomach. From the part of the vessel which lies within the liver and from its immediate subdivisions the posterior hepatics arise. A few small twigs also enter the dorsal side of the liver from the oesophageal ar-

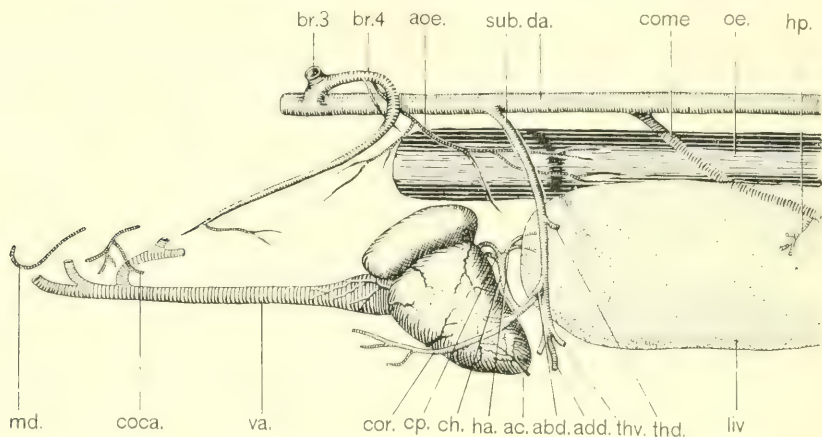


Fig. 1 Sketch to show the relations of coronary and hepatic arteries in *Scaphirhynchus*.

abd, artery to abductor muscle of pectoral fin
add, artery to abductor muscle
aoe, oesophageal artery
ac, arteria apicis cordis
br 3, br 4, third and fourth efferent branchial arteries
ch, common trunk of posterior coronary and ant. hepatic arteries
coca, coraco-arcualis (epigastric) artery
come, coeliaco-mesenteric artery

cor, coracoid artery
da, dorsal aorta
ha, hp, anterior and posterior hepatic arteries
liv, liver
md, mandibular artery
oe, oesophagus
sub, subclavian artery
thd, thv, thoracico-dorsalis and -ventralis arteries
va, ventral aorta

teries which are derived from the coeliaco-mesenteric and fourth efferent branchial arteries.

The anterior hepatic and posterior coronary arteries arise from a common trunk (*ch*) which in turn comes from the coracoid branch (*cor*) of the subclavian. This is the condition found in skates, which suggests that the vessels are homologous. Both the anterior hepatic and posterior coronary arteries, however,

are better developed in *Scaphirhynchus* than in the skates that have been described. The common trunk (*ch*) usually divides into coronary and hepatic divisions, each of which may again divide before entering its respective organ. The posterior coronary artery supplies the base of the heart and the aortic bulb; the anterior hepatic branches supply the very abundant lymphoid areas within the anterior part of the liver.

The apex of the heart may be supplied by a typical *arteria apicis cordis* (*ac*) such as Spalteholz ('08) found in certain turtles and lizards. This observation is of interest since Spalteholz thought the vessel did not occur in fish, birds or mammals. In the *Scaphirhynchus* used for the accompanying sketch the artery was as well developed as in the case of some of the turtles shown in Spalteholz's own figures. I have not been able, however, to demonstrate it in all cases, so the vessel is probably variable in its occurrence.

The points in which *Scaphirhynchus* departs from the typical condition found in skates are, briefly, the greater development of posterior coronary arteries with the associated anterior hepatic vessels, the absence of anterior coronaries, and the potentiality of an *arteria apicis cordis*.

POLYODON

The same hepatic and coronary arteries found in *Scaphirhynchus* are present in *Polyodon*, except for the *arteria apicis cordis* which has not been observed. The relation of these vessels to the heart and liver are essentially the same in the two species, but the origin of the hepatico-coronary trunk is quite different. In *Polyodon* this vessel arises from the fourth efferent branchial artery, a fact which it was found difficult to interpret when the arteries of this fish were first described.

This peculiar arrangement is easily explained by reference to the condition found in *Scaphirhynchus*. In the latter (fig. 1) there is an artery arising from the fourth aortic arch which supplies a part of the oesophagus and the region about the pericardium. In *Polyodon* this vessel has anastomosed with the he-

patie and coronary vessels and become their main trunk. The original hepatico-coronary trunk (*ch*) is now reduced to a small branch connecting the coronary artery with the subelavian. It was originally interpreted as merely a secondary anastomosis.

As in *Scaphirhynchus* no anterior coronary arteries are present. What might be interpreted as a rudimentary dorsal coronary is lost on the ventral aorta before reaching the heart. *Polyodon* differs from *Scaphirhynchus* chiefly in that the origin of the he-

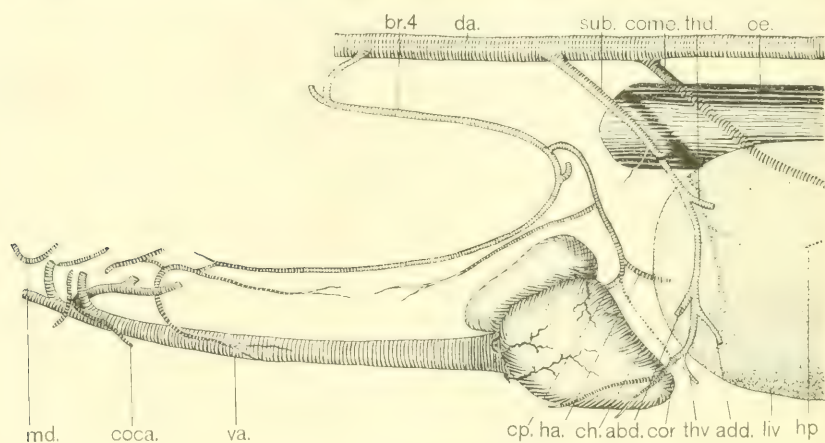


Fig. 2 The coronary and hepatic arteries of *Polyodon*. Letters as in figure 1.

patieo-coronary trunk is transferred from the coracoid branch of the subelavian to the dorsal part of the fourth efferent branchial artery.

LEPIDOSTEUS

In *Lepidosteus*, posterior hepatic and anterior coronary arteries alone are present. The latter arise from a vessel which comes off on the right from the large oesophageal trunk, which arises in turn from the subelavian artery. The anterior coronary arteries arise from a short median hypobranchial which derives its supply partly from the A. mandibularis (*md*) and partly from a commissural artery at the level of the second gill. These two vessels unite laterally and the resulting common trunk reaches

the median line below the aorta. The coronary arteries, which follow the aorta back, give rise to branches that may be definitely identified as ventral coronaries and others that may, with less certainty, be classed as dorsal coronaries.

Lepidosteus shows little in common with the preceding forms, but does present some similarity to the condition found in *Amia*.

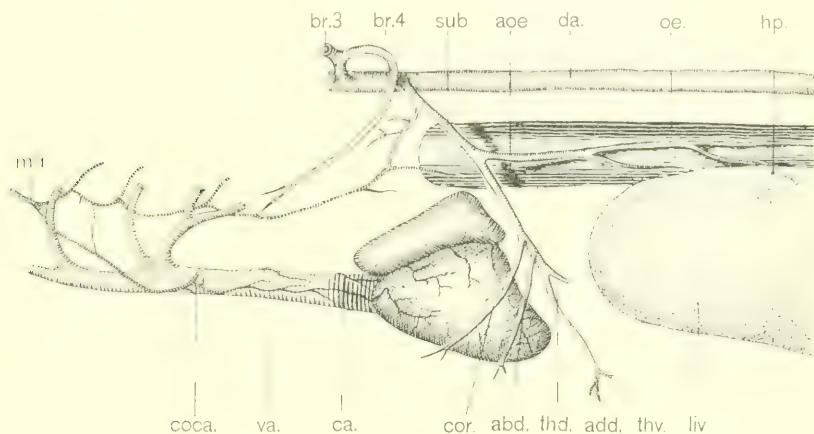


Fig. 3 The coronary and hepatic arteries of *Lepidosteus*. *ca.*, anterior coronary artery. Other letters as in figure 1.

AMIA

Like *Lepidosteus*, *Amia* has neither posterior coronary nor anterior hepatic arteries. The anterior coronaries arise from a median hypobranchial artery, as described by Parker and Davis ('99). The hypobranchial itself, however, may be derived, as shown in the figure, from two commissural arteries instead of the one usually described. Dorsal and ventral coronary arteries may be recognized in the branches that run along the upper and lower aspect of the aorta, but there seems to be no fundamental difference between them. The remaining arteries in *Amia* call for no special mention in this connection.

It will appear from the descriptions that there are among the ganoids two different schemes for supplying arterial blood to the heart and liver. One, represented by *Lepidosteus* and *Amia*,

approximates the teleostean type and presents no new points of special interest. The other, represented by *Scaphirhynchus* and *Polyodon*, appears to be a modification of the more primitive condition found in skates. Functionally, the latter arrangement would seem to be superior to the former, particularly in *Polyodon* where the whole supply to the heart and a large part of that to the liver is carried by a special vessel directly from the gills to these organs.

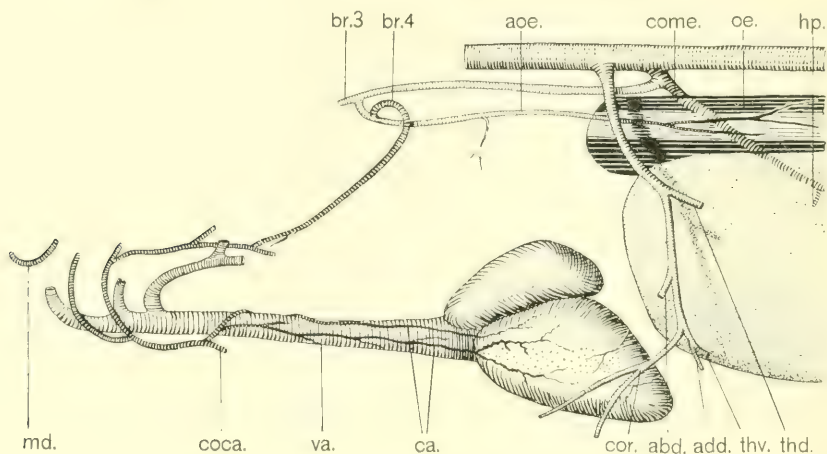


Fig. 4 The coronary and hepatic arteries of *Amia*. Lettering as in the preceding figures.

Morphologically, how much significance can be attached to these blood vessels is an open question. In different individuals of the same species of shark, Carazzi ('04) found the various arrangements of cardiac and oesophageal vessels that had been supposed to characterize several different species. The dissection of a few specimens of almost any fish will serve to show how greatly the vessels vary. On the processes involved in the production of such variations considerable work has been done. In other groups of animals blood pressure has been thought to play an important rôle in determining the interrelations of arteries (Thoma, '01). It seems probable to the writer that the distribution of blood vessels in the fish is primarily a functional

matter, dependent largely upon the interaction of blood pressure within the vessel and various forces that are brought to bear on its outer surface. Besides these passive factors, is the tendency of the vessel itself to spread into new regions.

This hypothesis receives some support from the results of experiments made on *Polyodon* and *Scaphirhynchus* in which the coeliaco-mesenteric artery can easily be tied.¹ There is, as above described, a normal anastomosis between the anterior and the posterior hepatic arteries thus connecting the branchial and intestinal arteries. There is also an anastomosis, smaller than the other, between the intestinal artery and a branch of the aorta that passes over the posterior side of the swim-bladder. Now when the coeliaco-mesenteric artery is tied off, the pressure must be lowered in the intestinal arteries and raised in all others. It would be expected that this would cause any secondary connections existing between the two systems to enlarge, and as a matter of fact a new artery adequate to supply the whole intestine is promptly formed through the posterior anastomosis. In neither form, however, have I been able to increase appreciably the caliber of the artery that passes through the liver, even by a second operation in which the new posterior mesenteric artery is tied. The difference in the behavior of the two anastomoses is very probably due to the fact that one occurs in the substance of the liver while the other is in the loose tissue of the mesentery.

A somewhat analogous set of interacting factors might easily determine the distribution of arteries in a growing embryo, and indeed, the evidence, so far as it goes, suggests that the arrangement of vessels is determined anew in each individual. That the same scheme tends to prevail in a given species or in related species is due, on this assumption, to the constant relations of

¹ Owing to its lack of scales *Polyodon* is especially favorable for this kind of experimentation. The procedure has been to open the abdomen by cutting along the linea alba and then turning the fish on its left side, when it becomes an easy matter to pass a ligature around the long free strand in which runs the coeliaco-mesenteric artery. The operation may be done without loss of blood. The fish usually recovers quickly and shows no bad after effects except that the cut in the linea alba is slow in healing. Several operated specimens were kept in the aquarium for three weeks before they were finally killed.

other organs rather than to an inherent morphological individuality of any given vessel. On this basis similar vascular arrangements might be produced by very different causes, and it is not impossible that this fact may explain the above described similarity between skates and the cartilaginous ganoids.

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THE CELL CLUSTERS IN THE DORSAL AORTA OF MAMMALIAN EMBRYOS¹

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TWO PLATES (11 FIGURES)

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I. GENERAL STRUCTURAL CHARACTERISTICS OF THE AORTIC CELL CLUSTERS

Figure 1 represents a portion of the ventral wall of the dorsal aorta as seen in a median sagittal section of a 9 mm. pig embryo. Along its endothelial surface may be observed five or six darkly stained cellular masses or clusters (ac_1-ac_5). Figures 2, 3 and 4 represent similar clusters as seen in transverse

¹ Some of the present observations were made while engaged in research work at the University of Strassburg and I wish here to express my indebtedness to Professor Weidenreich for the generosity with which the facilities of his laboratory were placed at my disposal. An abstract of the work was also published in the Proceedings of the American Association of Anatomists, Anatomical Record, Vol. 9, p. 77, 1915.

sections of the dorsal aorta. These figures illustrate the general appearance, maximum size, and anatomical relations of these structures. In consequence of their basophilic reaction to Giemsa's stain they stand out in sharp contrast to the red stained erythrocytes of the aortic circulation.

Cytological details in the structure of these clusters are illustrated in figures 6 and 9. The cells usually approximate a spherical form. The nuclei may be round but they more frequently present an indented or kidney shape and generally occupy an eccentric position in the cell body (figs. 6 and 9, m_1 , m_2). Usually, the nucleoplasm takes a lighter stain than that of the cytoplasm. Chromatin granules are rather evenly distributed throughout the nucleus. The cytoplasm takes a basophilic stain of varying intensity. Occasionally the cytoplasm is also vacuolated and contains phagocytized cellular inclusions, the latter being evidently chiefly of an erythrocytic nature. In some instances a reddish tinted or what appears to be a centrosphere is observed in the region of nuclear indentation (fig. 9, m_1). Aside from certain structural variations to be subsequently considered, the nuclear and cytoplasmic characteristics of the majority of these cells are comparable to the basophilic and phagocytically active cells or macrophags (mesamoeboids?) present in the circulating blood of the same embryos. That the component cells of these clusters become gradually dissociated and as detached cells contribute to the circulatory elements of the blood is indicated by those relationships in which certain cells are attached by only a slender cytoplasmic pedicle (figs. 6 and 9, m_1) and others are apparently entirely free (figs. 6 and 9, m_2).

These clusters were studied in pig, mouse and rabbit embryos and at certain developmental stages found in each of these mammals.

II. THE QUESTION OF THEIR ORIGIN

1. Statement of problem. In endeavoring to ascertain the nature and origin of these cell clusters in the aorta certain ques-

tions arise for consideration. Are they merely more or less agglutinated masses of circulatory blood corpuscles incidentally resting against the vascular surface or are they structures arising directly from the vascular wall? Furthermore, can their occurrence be correlated with any special developmental processes of the embryo?

Of preceding investigators who have recorded the observation of similar structures may be noted Maximow ('09, p. 157) for rabbit and cat embryos, Dantschakopff ('07) for the chick embryo and Minot ('12, p. 525) for the human embryo. Maximow and Dantschakopff interpret these cell masses as endothelial derivatives differentiating in situ from the vascular wall. Minot on the other hand did not find the evidence sufficiently convincing to justify the conclusion that they are of endothelial origin. Beyond the immediate question of their origin no data has been advanced as to why and under what conditions these cell clusters occur in the aorta. The present status of the problem and certain hematological questions associated with the subject are consequently such as to justify a further extension of the investigation.

Of the mouse, rabbit, and pig embryos studied in the present work the cell structures in question were found most pronounced and striking in the pig embryo. Consequently the following account is based chiefly on the results derived from the latter mammal, in which it appears that these clusters have not been previously described. The pig material for this purpose consisted of seventeen embryos varying in size from 6 mm. to 25 mm. fixed in Zenker-formalin (Helly's combination), embedded in paraffin or celloidin and the serial sections stained with Giemsa's fluid. Several of the 12 mm. embryos were also stained in toto with borax carmine and the sections counter stained with Lyon's blue.

2. *Grounds for regarding these clusters as of greater significance than merely incidental structures.* Upon first impression one may be inclined to discredit any special relationship between these cell masses and the aortic wall. As the result of further investigation it appears clear, however, that the phenomenon

is evidently one of greater significance than that of merely incidental cellular accumulations.

First, the clusters are in many cases at least, evidently rather firmly attached to the aortic wall. This is indicated by the fact that these structures may be found in the aorta even though the vessel is practically empty as may occur for example through the loss of blood during the preparation of the material. Again in some cases in which during the process of fixation almost the entire blood content settles toward one side of the aorta, the clusters instead of being carried along with the rest of the blood, continue attached in what appears to be their original position on the aortic wall. Finally, it may be observed that many of the larger cell masses present a rather elongated shape (fig. 1) with one end of the long axis adherent to the aortic wall and the other end free and directed caudalward (towards the left in the figure); i.e., the relations are such as might be expected in an attached cell mass one end of which was free to be carried down stream by the force of the blood current.

Second, the clusters are of constant occurrence in 6 mm. to 15 mm. embryos. Thus in a count for four 12 mm. embryos there was a total of 45 clusters distributed in the proportion of 11, 9, 13 and 12. On the other hand in embryos beyond about the 15 mm. stage, these cell masses are absent. Such conditions do not appear readily accounted for on the basis of accidental agglutination.

Third, in all the embryos studied the clusters were confined to the ventral half of the aortic tube and with greatest frequency toward the median region of its ventral wall (figs. 2, 3 and 4). In no case was a cluster found in the dorsal aortic wall. Such facts certainly appear indicative of a deeper relationship between these cell structures and the aortic tube.

In connection with this conclusion it is of course to be recognized that an occasional cell from the circulating blood may possibly now and then have been fixed in such a manner by the killing reagents as to be adherent to the vascular surface. On the other hand sections other than these passing through the base of the cluster may also present the deceptive appearance

of an absence of attachment. Finally with the gradual disappearance of the clusters in older embryos an intimate histological relationship with the aortic wall may become less and less evident.

3. *Evidence as to their origin from the vascular endothelium.* The preceding considerations rendered necessary a more detailed investigation of the cytological relations and origin of the aortic clusters. On the basis of the following results the conclusion is drawn that they are endothelial derivations and, as will be subsequently more fully elaborated, arise in relation to certain vascular conditions in the ventral portion of the aorta.

The first notable feature to which attention may be directed is the absence in the majority of cases of a definite continuity of the vascular endothelium in the region of contact between the clusters and the aortic wall. Cell boundaries are not clearly defined and the cells of the endothelium are in evidently syncytial relation (illustrated in figure 9 but not clearly evident in the low power drawings for figures 2 and 3). The cluster in figure 9 cannot be said to be resting upon a continuous sheet of typical flattened endothelium. On the contrary it can in the second place be shown that endothelium at the base of the cluster presents marked cytological modifications. In the vicinity of the cell masses the endothelial cells can be observed usually closer together than normally and the rounded nuclei frequently at one side present a more or less marked indentation or concavity giving it a kidney shaped appearance. (fig. 9). It is also to be noted that such endothelial conditions are most evident in the case of the clusters presenting the more intimate relationship with the vascular wall. Third, what appears to be transitional cytological changes can be traced from the somewhat lighter stained endothelial cells at the base of the cluster to the more sharply outlined and more deeply basophilic cells at the periphery of the mass (figs. 9, 6). It may also be observed in the same figures that many of the more peripheral cells still present a clearly defined cytoplasmic elongation or pedicle attaching them to the more central regions of the cluster. Fourth, that these conditions represent active cellular

differentiation rather than cellular disintegration appears demonstrated by the not infrequent evidence of mitotic activity as well as phagocytic function in the component cells of the clusters (fig. 9).

Fifth, areas of the aortic wall are found in which the endothelial cells present structural characteristics comparable to those already described in the endothelium adjacent to the larger cell masses (fig. 10a). Such areas are not, however, in any direct juxtaposition to the aortic clusters nor are they to be explained as deceptive appearances due to oblique or tangential sections of the endothelial surface. It may be observed that the endothelial cells are closer together, project above the general level of the vascular surface, and not infrequently take a more basophilic stain. The nuclei may be either rounded in form or approximate a kidney-shaped contour. No evidence was observed of mitotic cavity indicative of merely an incidental increase of endothelial cells in such regions through ordinary endothelial growth and cell multiplication. It is also noteworthy that such conditions were not found in the dorsal aortic wall as is illustrated in a comparison of figures 10a and 10b. Indeed the cytological structure and vascular relations of these cells appear identical with that of cells occurring in the aortic clusters. The fact that the aortic cell clusters present a great difference in size varying from the large masses in figures, 2 6 and 9 to these smaller accumulations of only a few cells and that they are also no longer present beyond certain stages of embryonic development, suggests that such areas as shown in figure 10a may represent end stages in the gradual dissociation and final disappearance of the aortic clusters, in which, however, there still remain indications of the endothelial reaction which has given rise to these structures. Sixth, of the two fixed tissue elements of the aortic wall which could possibly take part in the cell activities in question it is evidently primarily the endothelium rather than the mesenchyma which participates in the formation of these cell masses. The demarcation between the mesenchyma and the aortic clusters is fairly well defined, nor was there obtained any conclusive evidence of a

possible migration of free cells from the mesenchyma into the clusters.

Finally, it is important to note that not infrequently the aortic clusters are in direct relationship or continuity with cell masses situated within certain atrophying arterial branches of the aorta. These intra-arterial masses have evidently arisen *in situ* from the endothelium of the artery in question and as described elsewhere (pp. 409-411) probably constitute the primary source of origin of the aortic clusters.²

III. CONCERNING THE CORRELATION OF THE CLUSTERS WITH CERTAIN AORTIC DEVELOPMENTAL PROCESSES

1. *Degeneration and caudal wandering of aortic rami.* In the development of the mammalian aorta there occur two important vascular changes involving a shifting or caudal wander-

² An additional observation which may be conveniently recorded here relates to a type of structure illustrated in figure 5. This group of cells is attached to the ventral aortic wall and projects into the lumen of the vessel but differs from the typical aortic cluster through its enclosure by a more or less definitely marked peripheral membrane (*en*). In close relation to the membrane are a number of cells some of which present the flattened endothelial form while others are more rounded in shape. In other respects the component cells appear similar to those of the clusters. Such structures are apparently of rare occurrence for in the present material they were found in only one embryo, a 12 mm. specimen (W. U. coll. No. 3), in which there were two of these bodies, both ventrally located. (This embryo had been stained with borax-carmin and the present statements are made without having data derived from Giemsa stained material.) It is of interest to observe that this same embryo was also deficient in the usual number of aortic clusters, for only three of the latter were found instead of the 9-13 clusters in each of four other embryos of the same size.

The aorta of the same embryo also contained three elongated cellular strands evidently of endothelial nature. Two of these strands (about 60 micra in length) were attached to the left umbilical artery near its origin from the aorta. The third strand about 300 micra in length and varying from one to several cells in thickness, was connected by only a slender cytoplasmic strand to the aortic wall. Two such structures were also found in a second 12 mm. embryo (W. U. coll. No. 5), one in the aorta and the other in the region of origin of the umbilical arteries. Nothing conclusive was ascertained as to the significance of these strands, but the suggestion merits further investigation as to whether they may possibly be associated with the fusion of the two original dorsal aortae. With reference to our present purpose, however, it is of interest to note that many of their component cells present rounded form, kidney shaped nuclei, and phagocytic activities comparable to that of the component cells of the aortic clusters.

ing of certain arterial branches of the aorta and an extensive degeneration of others.

Directing attention first to the degenerative changes it will be recalled that the early embryonic aorta has three sets of eighteen to twenty or more paired branches - dorsal, lateral, and ventral. Of these rami, nearly all the dorsal vessels persist throughout embryonic development, whereas practically all the remaining vessels, with certain exceptions, subsequently atrophy and disappear. Thus in the case of the human embryo it has been shown that the primitive lateral branches of the aorta which form an extensive system in the twenty-three somite embryo, have in the 16 mm. to 19 mm. stage embryos become largely atrophied. The single median arterial stems which have replaced the extensive series of paired ventral arteries of younger stages (Tandler, '03) and in a 5 mm. embryo extend as a "complete series of unpaired or median ventral segmentals from the seventh cervical to the second lumbar segment inclusive," in a 7 mm. embryo "have been reduced to three main trunks" the coeliac, superior, and inferior mesenteric arteries. (Keibel-Mall, '12, pp. 603, 611, 643, 653).

Second, it is to be observed that the three remaining arterial trunks to which the ventral segmental aortic arteries have been reduced, undergo a remarkable shifting or caudal wandering as first described by Mall in 1891 and subsequently confirmed by Tandler ('03) and Broman ('08). In the human embryo for example "the coeliac artery thus wanders from the seventh cervical to the twelfth thoracic segment, a displacement of some eleven segments, and the superior mesenteric artery almost equally as far (ten segments, second thoracic to third lumbar); whereas the inferior mesenteric artery wanders through but three segments (twelfth thoracic to third lumbar)" (Evans '12, p. 647).

Referring again to the human embryo it is of especial interest with reference to our present purpose to note that all of these vessels "usually attain the adult levels by the time the embryo is 17 mm. long." Furthermore this caudal wandering of the intestinal arteries is not by a displacement of the aorta on the

vertebral column, but is an actual shifting of these ventral branches when compared with the dorsal branches of the same trunk (Evans, '12, p. 648).

Although the details have not, so far as I am aware, been as carefully ascertained as in the case of the human embryo, essentially the same conditions evidently maintain for the pig embryo as in other mammals. The ventral and lateral aortic rami undergo a similar degeneration. The shifting of the ventral or intestinal vessels is indicated by the fact that the coeliac artery which in a 6.5 mm. pig embryo is at the level of the eighth cervical segment, in a 12 mm. embryo is at the level of the fifth thoracic segment. Again, the superior mesenteric artery in the 6.5 mm. embryo is at the level of the third thoracic segment, but has descended to the level of the eighth thoracic segment in the 12 mm. embryo. Both the atrophy of vessels and caudal wandering appear practically complete at about the 15 mm. stage.

2. Correlation of the aortic clusters with these vascular changes.

In a comparative analysis of the preceding data for the vascular changes and cell clusters in the aorta certain striking relationships become evident. First, both phenomena occur within the same period of embryonic development—between the stages of about 5 mm. to 15 mm. in the pig embryo. Again, both the formation of the clusters and the degenerative changes and caudal wandering of the arteries, as already indicated, are confined to the same region of the aorta, namely, its ventral wall. Third, in a linear direction within this ventral region the cell clusters are furthermore fairly evenly distributed between the coeliac and umbilical arteries as shown in the following data for four 12 mm. embryos:

| SPECIMEN | (W. U. COLL. NO.) | NUMBER OF CLUSTERS BETWEEN: | | TOTAL NUMBER OF CLUSTERS |
|----------|-------------------|--|--|--------------------------|
| | | The coeliac and superior mesenteric arteries | The superior mesenteric and umbilical arteries | |
| 1 | (4) | 5 | 6 | 11 |
| 2 | (1) | 3 | 6 | 9 |
| 3 | (2) | 7 | 6 | 13 |
| 4 | (5) | 5 | 7 | 12 |

Fourth, there are certain important cytological conditions to be considered in the degenerating arteries themselves. Many of these vessels are found compactly filled with basophilic staining cells (cf. figures 1 da, 7 and 11). Such conditions are found near the aortic origin of the artery and may extend for short distances into the ramus, occasionally continuing to a point where the degenerating vessel is lost in the mesenchyma. Erythrocytes are strikingly deficient in such regions and may indeed be entirely lacking throughout the vessel, a condition evidently indicative of the reduction if not complete cessation of the circulation through these retrograding arteries. The component cells of these intra-arterial cell masses may take a somewhat lighter stain but they otherwise appear cytologically identical with those of the aortic clusters. They are phagocytically active (fig. 11, in) and undergo cell multiplication (fig. 7, d). In regions of the artery not thus occluded the endothelial cells are frequently rounded up or swollen and project into the lumen of the vessel. The transitional stages to be found between the still intact endothelial cells and the intra-arterial masses seem to leave no doubt but that the latter have arisen in situ from the lining endothelium of the retrograding vessel. Finally, there remains the crucial fact of an intimate relationship between these intra-arterial masses and the aortic clusters. This is illustrated in figure 6 in which the intra-arterial cell mass (*iam*) when followed toward the aorta is found to terminate in an aortic cluster situated within the lumen of the aorta. It may be observed that the component elements merge into each other with no evident line of demarcation between them. Indeed the cytological conditions and morphological relations are such as to justify regarding the phenomenon as essentially comparable to a partial evisceration of the contents of the degenerating artery into the lumen of the aorta. Such a relation of aortic clusters and intra-arterial masses is of frequent occurrence. It is also noteworthy that in many cases where such a relationship is apparently lacking the aortic cluster is, however, situated in a well marked depression or concavity in the aortic wall, and that some of these depressions are in relation

to the atrophied remnant of a small artery which soon terminates blindly in the adjacent mesenchyma. (Such depressions are inadequately shown in *ac2* and *ac4* of figure 1, but can be readily demonstrated in serial sections.) Not infrequently in instances where such depressions are lacking there may still be observed a clearly evident irregularity, sometimes of a more or less whorled character, in the arrangement of the mesenchymal cells at the base of the cluster as compared with the adjacent regions of the aortic wall (figs. 2, 3, *s*). Occasionally the clusters occur in pairs (fig. 4) as if they had arisen in connection with the simultaneous atrophy of two paired aortic rami. In apparent corroboration of these results certain conditions are occasionally found at the aortic entrance to an as yet relatively intact arterial ramus in which the cytological structure, form and relations of the component elements of the vascular surface suggest an early stage in the endothelial activities involved in the production of the intra-arterial and aortic cell masses (fig. 8).

On the basis of the preceding data the conclusion is drawn that the formation of the cell clusters in the aorta are not only intimately associated with, but are also evidently correctly interpreted as a direct result of the developmental processes involved in the atrophy of the ventral and lateral aortic rami and the establishment of the permanent intestinal arteries of the adult organism.³

³ Concerning the remarkable caudal wandering of the visceral rami of the aorta a number of hypotheses have been advanced to account for the phenomenon (Broman, '08, Tandler, '93), Evans, '12) but the exact manner in which the process takes place has apparently as yet not been established. In connection with the present study it may be observed that the depressions in the aortic wall and the relations of the atrophied arterial stems and the aortic clusters are such as to suggest that arterial remnants of the former aortic rami are ultimately incorporated into the aorta itself. Such additions and consequent inequalities of growth in the ventral region of the aortic wall as contrasted with its dorsal portion may in a final solution of the problem be found to contribute materially to the caudal shifting of the coeliac and mesenteric arteries. Evans ('12, p. 649) does indeed express the opinion that a primary factor in these vascular changes is an unequal growth of the dorsal and ventral walls of the aorta but has not, so far as I am aware, elaborated the specific nature of the process.

IV. DISCUSSION CONCERNING ENDOTHELIAL TISSUE AS CONTRIBUTING TO THE CELLULAR ELEMENTS OF THE BLOOD

On the basis of the present results it appears evident that the cell clusters in the embryonic aorta furnish an instance of the vascular endothelium contributing cellular elements to the circulating blood. Since this is not entire agreement with one of the postulates of the angioblast theory, namely that all the blood cells of the organism are direct descendants from the early embryonic blood islands, it becomes of interest to note the conditions under which this endothelial activity is taking place. The close association of the aortic clusters with degenerating vessels directs attention to the occurrence in these retrogressive vessels of stimulative factors to which the endothelium reacts in the manner under consideration. As stated by Thoma ('93) and elaborated by Mall ('06) in the case of the embryonic liver, a vessel in which there is a reduction of the circulation below normal tends to shrink and disappear. The occurrence of such a retardation of circulation in the atrophy of the aortic rami is indicated by the marked absence of red blood corpuscles (p. 410). With the circulation practically at a standstill it is not improbable that diminished oxidation and inhibition of gaseous interchange are in part at least productive of mildly abnormal chemical and toxic conditions conducive to the phagocytic activities, endothelial proliferation and consequent formation of intra-arterial cell masses and aortic clusters.

In support of this conclusion attention may be called to the emphasis being more recently attached to such abnormal intra-vascular conditions as stimulative to endothelial activity. Thus Mallory ('00 and '14, pp. 165-166, 183) advances grounds for the conclusion that certain dilute and weak toxins stimulate endothelial proliferation and phagocytes and maintains that in this manner arise the macrophages encountered in many diseases. Batchelor ('14) and Scott ('14) record marked proliferative endothelial changes and phagocytic activity in hepatic vessels occluded by artificially produced emboli and wounds.

Finally the experimental results of a number of investigators of whom may be mentioned Tschaschin ('13, p. 370) and Mac Curdy and Evans ('12, p. 1695) may be adequately summarized in the recent statement by Evans ('15, p. 254) "that occurrences which place the endothelium of the most various vessels under conditions, such, for instance, as a direct injury of the endothelium, cessation of the adjacent current, in short in all cases of thrombosis or embolism, lead to the proliferation of endothelium" "Probably no area of the body can be excluded in this respect."⁴

In conclusion, therefore, it may be stated that while the original assumption of the angioblast theory—that vascular endothelium does not give rise to cellular elements of the blood—may under normal conditions be true for the general systemic vascular system, it appears that in both embryo and adult mammals, endothelial tissue ordinarily passive may under certain abnormal conditions, however, assume proliferative activities contributing to the free cellular elements of the circulating blood.

V. RÉSUMÉ

1. During the development of mouse, rabbit, and pig embryos certain well defined cell masses or clusters are found in the aorta of these mammals.

2. The majority of the component cells of these clusters are in their cytological characteristics comparable to the basophilic and phagocytically active cells or macrophags (mesamoeboids?) in the embryonic circulation.

3. Their constancy of occurrence, firm attachment and restriction to the ventral wall of the aorta indicate that these cell clusters are not merely chance cellular accumulations but structures having a significant relationship to the vascular conditions in the aortic artery.

⁴ The participation of the mesothelium in the origin of macrophags in the embryonic coelom (Emmel, '15) is not improbably also a reaction to stimulative conditions arising in part at least through degeneration and disintegration of erythrocytic and other foreign elements escaping into these cavities.

4. Certain structural modifications and frequent discontinuity in the endothelium at the base of these masses, the cytological characteristics transitional between these endothelial cells and the component cells of clusters, the evidence of mitotic activity, variation in size, and relationship to certain degenerating aortic rami, support the conclusion that the aortic clusters have arisen from the vascular endothelium.

5. An intimate association and fundamental causal relationship can be demonstrated between the formation of the aortic clusters and the developmental processes involving the atrophy of certain aortic rami and the establishment of the permanent intestinal arteries of the adult mammal. The endothelium in degenerating stems of the aortic rami is stimulated, (evidently through certain toxic conditions arising in the retrogressive vessels), to phagocytic and proliferative activities giving rise to infra-arterial cell masses constituting a primary source of origin of the aortic clusters.

6. On the basis of the cumulative evidence of various recent investigators it appears evident that the original assumption of the angioblast theory that the endothelium of the general systemic vascular system does not contribute to the cellular elements of the blood, while possibly true under normal conditions, requires the qualification that under certain abnormal conditions endothelial tissue ordinarily passive may in both embryo and adult assume such proliferative activities.⁵

⁵ While the present paper was in press an article appeared in the *Anatomical Record*, Vol. 10, p. 417, by Jordan on the "Evidence of Hemogenic Capacity of Endothelium." It is of especial interest to note that Jordan records the observation of cellular structures in the aorta of mongoose and turtle embryos apparently similar to the clusters occurring in the pig, mouse and rabbit embryos. Here again the clusters are confined to the ventral region of the aorta. In the mongoose and turtle, just as in the pig, "the clusters show a progressive increase in size corresponding with the age of the embryos, between 5 and 10 mm., indicating an intrinsic growth" p. 419. It is emphasized that "similar clusters are found nowhere else either in the yolk sac or the embryonic vessels or sinusoids" (p. 418) and in agreement with the results of the present paper cogent reasons are advanced for regarding these clusters as being not merely chance cellular accumulations, but as structures arising from the vascular endothelium.

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PLATE I

EXPLANATION OF FIGURES

Figures 1, 6 to 11, inclusive, are from 9 mm. pig embryos fixed in Zenker-formalin and stained with Giemsa. Figures 2 to 5, inclusive, are from 12 mm. embryos fixed in Zenker-acetic and stained with borax carmine and Lyon's blue. All figures are from sagittal sections, except figures 2 to 5 which are from transverse sections of the aorta. The drawings, reduced one-fifth in reproduction, were originally made with the magnifications obtained in the following combinations of Zeiss apochromatic lenses and compensating oculars:

Figs. 1 to 4, oc. 8, No. 3, obj.

Fig. 5, oc. 4, 2 mm. obj.

Figs. 6 to 11, oc. 6, 2 mm. obj.

Recognition is due the artist, C. D. Jarrett, for faithful reproduction of cytological details.

ABBREVIATIONS

| | |
|---------------------------------------|---|
| <i>ac</i> , aortic cluster | <i>la</i> , lateral aortic ramus |
| <i>d</i> , mitosis | <i>m</i> , the more highly differentiated basophilic cells (or macrophags) in the aortic clusters |
| <i>da</i> , degenerating aortic ramus | <i>r</i> , erythrocyte |
| <i>dw</i> , dorsal aortic wall | <i>s</i> , mesenchyma |
| <i>e</i> , endothelium | <i>va</i> , ventral aortic ramus |
| <i>iam</i> , intra-arterial cell mass | <i>vw</i> , ventral wall of the aorta |
| <i>in</i> , phagocytic inclusion | |

The direction of circulation and the long axis of the aorta is indicated by an arrow.

A portion of the ventral region of the aorta as seen in longitudinal section, showing the general appearance and morphological relations of the aortic clusters (*ac*₁-*ac*₅). A caudalward projection of the free end of the clusters is illustrated in *ac*₄. Cluster *ac*₅ is situated at the entrance to a root of the superior mesenteric artery. *da* is a section of a degenerating aortic ramus packed with basophilic cells similar to those of the clusters.

2 and 3 Show the form and structural relations of the aortic clusters as seen in transverse sections of the aorta. Adjacent to the base of these clusters may also be observed a variation in the general arrangement of the mesenchymal cells (*s*).

4 Illustrates the double aortic clusters occasionally found.

5 One of two spherical masses of cells found in a 12 mm. embryo. The mass appears in some respects comparable to the aortic clusters, but is surrounded by a more or less definite endothelial membrane (*en*). Situated in the ventral wall of the aorta.

6 Section of an aortic cell cluster situated at the entrance to a ventral aortic ramus and in intimate relationship with the intra-arterial cell mass (*iam*) filling the latter vessel. Also illustrates the more definite differentiation of the peripheral cells (*m*₁) of the clusters, some of which appear entirely detached from the main mass (*m*₂).

7 Section of an intra-arterial cell mass in a degenerating ventral aortic ramus. Note absence of a definite lining endothelium and the evidence of active cell multiplication (*e*).

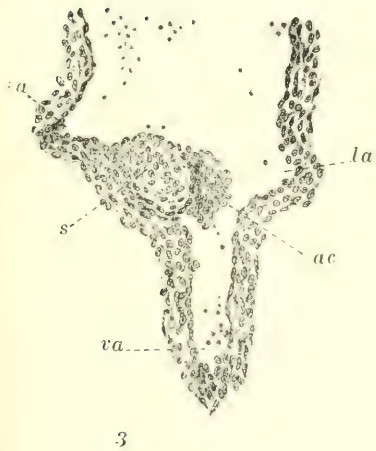
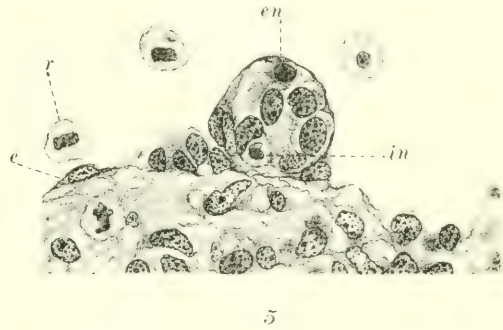
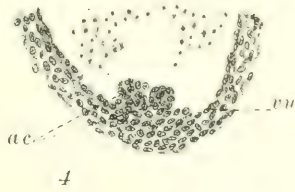
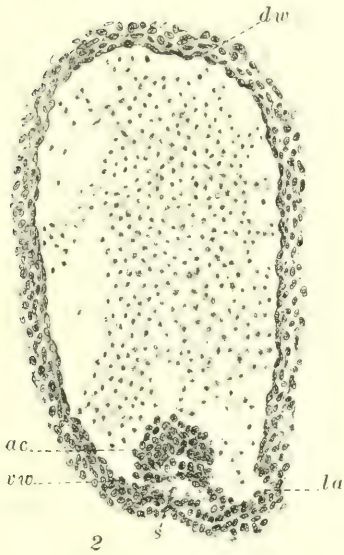
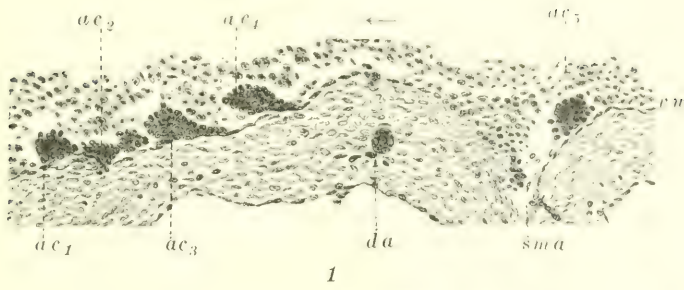


PLATE 2

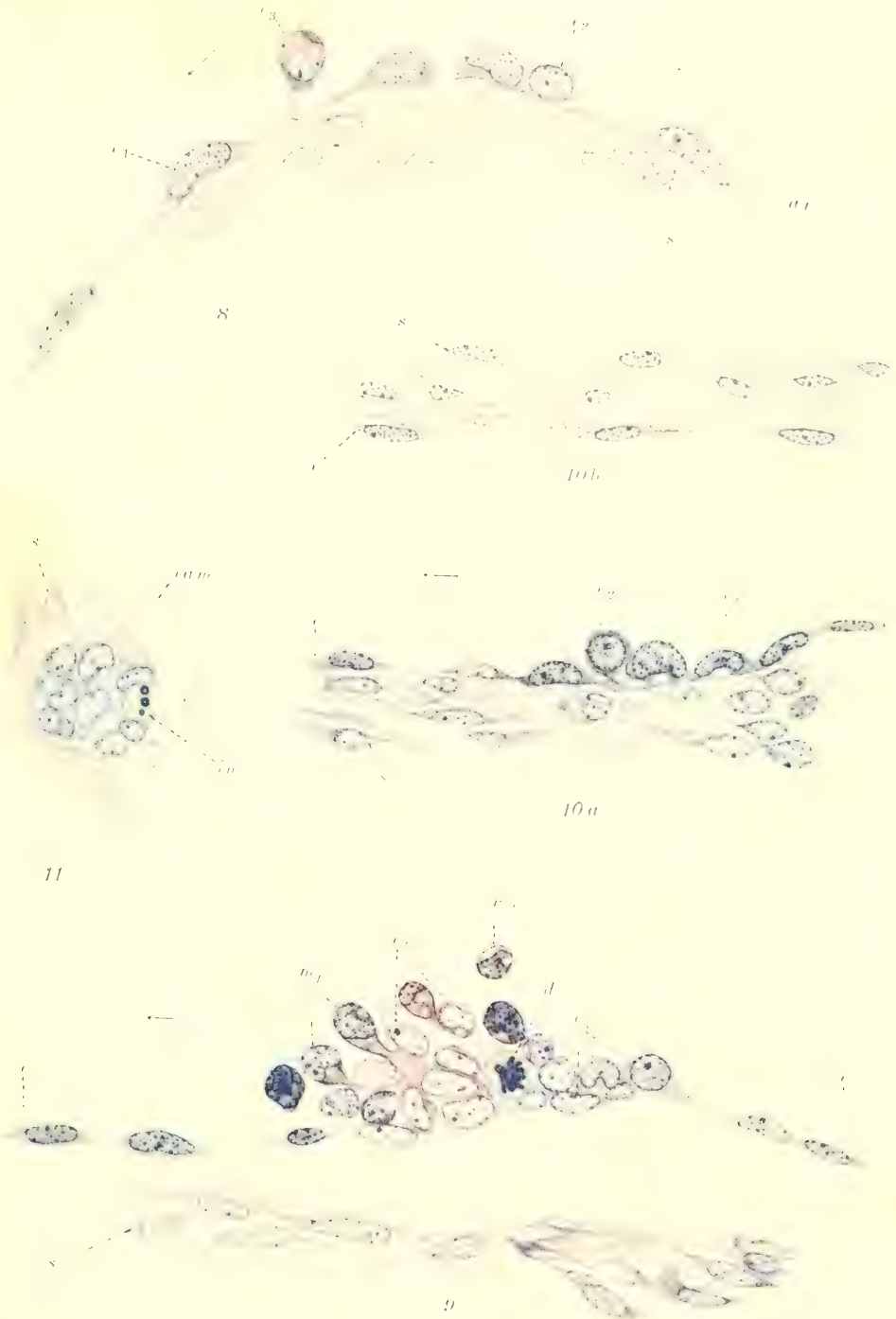
EXPLANATION OF FIGURES

Region of the vascular endothelium at the entrance to an aortic ramus (*ar*). Is of interest as showing cytological changes in the endothelial cells (*e*₁, *e*₂, *e*₃) suggestive of an initial stage in the formation of a cell cluster. The drawing includes only the caudal side of the orifice of the smaller vessel, the entrance to which is indicated by the arrow at the right.

9 Illustrates the cytological structure of an aortic cluster, the discontinuity of the aortic endothelium at its base, evidence of mitotic cell multiplication (*d*), the gradation in structural characteristics from the basal (*e*₁) to the more peripheral cells of the cluster (*e*₂, *m*₁) and the apparent detachment of some of the latter as free cells (*m*₂). The anatomical relations appear brought out to advantage in this particular case, through the artificial separation of the endothelial surface (*e*) from the underlying mesenchyma (*s*) during the histological preparation of the material.

10a and 10b Are respectively from directly opposite regions of the ventral and dorsal walls of the aorta and illustrate striking differences in endothelial structure. Note the flattened form and much wider separation of the endothelial cells in 10b as contrasted with 10a and, in the latter case, the kidney shaped nuclei and rounded cells (*e*₁, *e*₂) raised above the general level of the vascular surface.

11 Transverse section of a ventral arterial branch of the aorta filled with basophilic cells. Some of the cells are phagocytically active (*in*). A definite lining endothelium is no longer evident. (cf. fig. 7 and fig. 1, *da.*)



THE GENERAL FUNCTIONAL SIGNIFICANCE OF MITOCHONDRIA¹

E. V. COWDRY

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Years ago Schultze defined protoplasm as being a glass-like, semifluid material in which granules are embedded. Only now are we beginning to see more clearly, for investigators have succeeded, in the last few years, in separating from this baffling heterogeneous complex a class of granulations which are more or less distinct chemically, morphologically and physiologically and which occur in almost all protoplasm. These granules are usually called 'mitochondria.' They are not a recent discovery for both Flemming and Altmann observed and described them, but recent studies have enabled us to define them more accurately and have in consequence forced us to revise our views of cell structure.

MORPHOLOGY AND NOMENCLATURE

Mitochondria vary in form from granules (0.2μ - 2μ) to rods and filaments, which may be straight, curved or even forked. Sometimes they are ring-shaped, pear-shaped and possess bleb-like swellings. Networks rarely occur. That mitochondria collectively undergo changes in form was early recognized through the study of successive stages of histogenesis, particularly of spermatogenesis. But it remained for the Lewises ('15, p. 352) to actually follow the form changes of individual mitochondria by the observation of living cells in tissue cultures, to see filamentous mitochondria changing to granular ones, etc.

The Lewises also observed that the mitochondria moved freely, and quite rapidly, from place to place in the cytoplasm.

¹ Aided by the Carnegie Institution.

Of course it does not follow that they behave in the same way in the cells in the organism, but the observation nevertheless strengthens our suspicions of the ancient hypothesis of a protoplasmic reticulum, which still persists in the form of misleading diagrams in all our textbooks of histology.

Following along the lines of Maximow's ('13, p. 244) studies N. H. Cowdry has observed the changes in the shape of mitochondria in the streaming protoplasm of living plant cells. He has seen filaments assume the form of loops and spirals in response to currents and eddies in the stream indicating clearly that they are flexible and that their form is in a measure determined by their environment.

In spite of observations such as these the literature is still befogged by the use of a specific term for each of the different forms of mitochondria. Thus, some would restrict the term 'mitochondria,'² which was originally applied to them, to granules only; when the granules are arranged in rows they would be called 'chondriomites'; the filaments, 'chondriocontes'; the word, 'chondriosomes,' would be used as a generic term to include all the forms: and, finally, the cytoplasmic content of mitochondria would be styled the 'chondriome.' This system of terminology was very popular for a few years. It was partially supplanted by the short-lived 'Plastochondrial' nomenclature advocated by Meves ('10, p. 150), the chief objection to which is that it was devised to proclaim the view that mitochondria play an important part in histogenesis. American investigators have, with few exceptions, from the beginning employed the term 'mitochondria,' exclusively, recognizing well that the same material, under different conditions, may assume special forms. Even the word 'mitochondria' leaves many things to be desired, but it is in general use, it is descriptive of morphology only and it does not commit the user to any hypothesis of the functional significance of the materials in question. True, we cannot use the name in the exact sense that Benda, who introduced it, used it no more than we can employ the term 'cell' with anything like its original meaning.

² From *mitros*, a thread and *χόνδρος*, a grain.

Yet no one would invite us to give up the word 'cell' and to substitute a new and more appropriate term in its place.

Mitochondria may be provisionally defined as substances which occur in the form of granules, rods and filaments in almost all living cells, which react positively to janus green and which, by their solubilities and staining reactions resemble phospholipins and, to a lesser extent, albumins.

RELATION OF MITOCHONDRIA TO OTHER CYTOPLASMIC CONSTITUENTS

It must again be emphasized that mitochondria are by no means a recent discovery. Altmann and Flemming undoubtedly observed and described them, but Altmann grouped other structures, like secretion granulations, under the same heading of 'bioblasts' just as Flemming did with his 'fila.' The interstitial granules of Koelliker and the plasmosomes of Arnold as well as the cytomicrosomes of Heidenhain are also in part mitochondria.³ What is new about mitochondria is that they are without doubt a more concrete class of cell granulations than any of the above mentioned. Investigators were not slow to appreciate the value of the work of the older authors, in fact the confusion which is so woefully apparent, at the present time, in our ideas of the relation between mitochondria and other formed bodies is due to the fact that they went altogether too far in identifying mitochondria with previously described cytoplasmic constituents to which they are in no way related. This tendency to carry things to an extreme, to overstep the mark completely, is manifested in the study of mitochondria over and over again.

A case in point is that of the chromidial substance. Goldschmidt ('09, p. 107) and his pupils have persistently asserted that mitochondria belong to the category of the chromidial

³ One must be on the lookout for descriptions of mitochondria under the following headings also: chondriospären and chondriorhabden (Benda), neurosomes (Held) karyochondria (Wildman), fuchsinophile granules, plasmafaden (Retzius), plastidulen (Maggi), perinème and pericaryonime (Renaut), substantia granulo-filamentosa, etc.

apparatus. Duesberg ('10, p. 652) and I ('12, p. 497), among others, have shown that they are two separate and distinct substances. The wonder is that they could ever have been confused for we have ample evidence that the chromidial substance (Nissl substance) is a nucleoprotein containing iron (Scott '05, p. 507), formed at least in part through the activity of the nucleus, and the mitochondria a phospholipin albumin complex. In this connection, also, must be mentioned the attempts of Bouin ('05, p. 917) and others to identify mitochondria with the previously discovered 'ergastoplasme' (protoplasme supérieur, kinoplasme, archoplasme, etc.). The term 'ergastidions' which Laguesse ('11, p. 276) used for some years instead of mitochondria, and later abandoned, is a relic of this tendency. Regaud and Mawas ('09, p. 229) have vigorously combatted the view that the mitochondria and ergastoplasme are identical and the justice of their arguments is apparent when we remember that the terms of 'ergastoplasme' and 'chromidial substance' are usually applied to one and the same material.

Another instance is that of the reticular apparatus of Golgi⁴ which Hoven ('10, p. 479), Rina Monti ('15, p. 40) and others believe to be, in some cases, identical with mitochondria. But here the question is a far more complicated one, because it is still impossible to define the reticular apparatus in any other terms than in the appearance of cells fixed and stained by notoriously capricious methods of technique. The relation between the two substances cannot be profitably discussed before refinements in technique are made and we learn more about the reticular apparatus.

CHEMISTRY

It is an interesting and rather unusual occurrence, in the study of mitochondria, for three independent lines of investigation to yield similar results, yet Regaud ('08, p. 720), in the first place, in the study of mammalian tissues; Fauré-Fremiet ('10, p. 622), who worked on protozoa; and the botanist, Löwtsch (1913, p.

⁴ Synonyms: apparato reticolare interno, binnennetz (Kopsch), netzapparat, saftkanalchen and trophospongium (Holmgren)? spiresmes (Nelis)? conduite de Golgi-Holmgren (Cajal)? canalicular apparatus? etc.

203; '14, p. 269), have all arrived at the conclusion that mitochondria are chemically a combination of phospholipin and albumin, which, in itself, speaks very strongly in favor of the unity of the class of granules under consideration. The evidence is briefly this:

1. Mitochondria are almost completely soluble in alcohol, ether, chloroform and dilute acetic acid. They are rendered insoluble by chromization. They are not doubly refractile and they do not stain with Sudan III or Scharlach R. They are only sometimes blackened with osmic acid.

2. It is said that part of the mitochondrial substance is not soluble in these fat solvents and it is supposed that this portion is albumin (see also Bullard '16, p. 26) for formalin and bichromate, which are used as fixatives for mitochondria, are energetic coagulants of albumin. Millon's reagent is the only color test for protein which can be applied to material in sections. So far as can be ascertained⁵ it is negative, but this cannot be stated positively because, even if there were a change in color, it might not be of sufficient intensity to be appreciated in filaments of such extreme fineness as mitochondria (0.2μ in diameter) embedded in a colored cytoplasm. Mitochondria do not give any of the color reactions of polysaccharides.

3. Artificial mitochondria have been made by Löwschin of lecithin, and albumin solutions (resulting in the formation of lecithalbumin?) which apparently present the same form and solubilities as true mitochondria. They form granules, rods and filaments which multiply by division. He embedded them in glycerin-gelatin, fixed them and found that they stained in the usual way by the various mitochondrial methods.⁶

This evidence is good (being apparently accepted by the Kochs '13, p. 427 and Mathews '15, p. 102, as far as the phospholipin fraction is concerned) but it cannot be considered as

⁵ Bensley, personal communication.

⁶ Mayer, Rathery and Schaeffer ('14, p. 612) have been able to alter the mitochondria experimentally in liver cells. In stages with more mitochondrial substance chemical analysis shows an increase in phosphorized lipid; in stages with less, a diminution.

absolutely conclusive because it is subject to all the multitudinous objections, which are very justly raised, against the results of analyses of intracellular material. As yet no direct chemical analyses of mitochondria have been made. The eggs of fishes may prove favorable material because they are fairly large, and, since their cytoplasm is quite liquid, the mitochondria can be easily collected in a compact mass to one side by centrifuging, and, perhaps, be dissected out or removed by means of a capillary pipette.

Hoppe Seyler pointed out that lecithin (a typical phospholipin) and cholesterol are to be found almost anywhere that life phenomena exist. In fact a great wave of revived interest is manifested in recent chemical and pathological literature in these complex compounds of fatty acid, phosphorus and nitrogen. Mathews very aptly says that the phospholipins are the most important substances in living matter:

For they are found in all cells, and it is undoubtedly their function to produce, with cholesterol, the peculiar semifluid, semisolid state of protoplasm. The latter holds much water in it but it does not dissolve. Indeed it may be said that the phospholipins with cholesterol make the essential substratum of living matter.—This physical substratum of phospholipin differs in different cells and probably in the same type of cells in different animals, but everywhere, from the lowest plants to the highly differentiated brain cells of mammals and of man himself, it possesses certain fundamental chemical and physical properties. In all cases the phospholipin substratum is soluble in alcohol containing some water. ('15, p. 88).

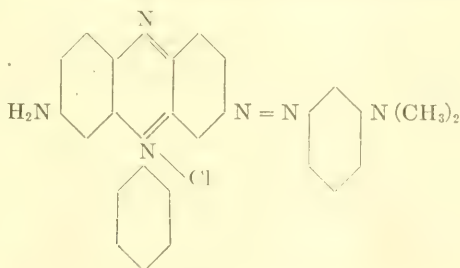
In view of these considerations it is interesting to enquire whether the distribution of mitochondria in cells corresponds with that of the phospholipins. It is certainly true that mitochondria are more widely distributed than any other kind of cytoplasmic granulation now known to us. They occur in almost all cells. Certain cells, like the fully differentiated non-nucleated red blood cell, undoubtedly contain a large amount of phospholipin though no formed mitochondria can be seen. The mitochondrial substance is probably present in solution (Cowdry '16), because it would be obviously absurd to state that it must always occur in a certain state of condensation which makes it visible with the microscope.

Chemically, then, we may for the present regard mitochondria as being a combination, in varying amounts, of phospholipins and protein. The phospholipins probably differ in quality as well as in quantity and this is in all likelihood the case with the protein also. It is probably the chemical basis of the perplexing differences in solubility and staining reaction, and to a lesser extent, of the differences in form, which mitochondria exhibit in different cells.

THE JANUS GREEN REACTION

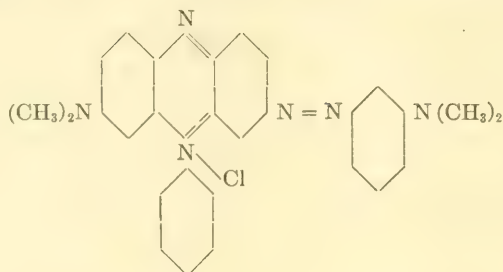
That mitochondria in living cells stain specifically with janus green was originally discovered by Michaelis ('99, p. 565). Furthermore, when the janus green is reduced by the tissue, a red diethylsafranin is formed which also colors the mitochondria specifically. The delicacy of the reaction is shown by the dilution of the stain which will give it. I have found that mitochondria will stain in human lymphocytes in a dilution of janus green in physiological saline of 1: 500,000. This is very remarkable when one reflects that the mitochondria are only about 0.2μ in thickness. But the most significant fact is that the reaction depends upon the presence of two ethyl groups in the safranin portion of the janus green molecule. There are three janus greens of the following formulae:⁷

1. Janus green (Grübler) safraninazodimethylanilin chloride

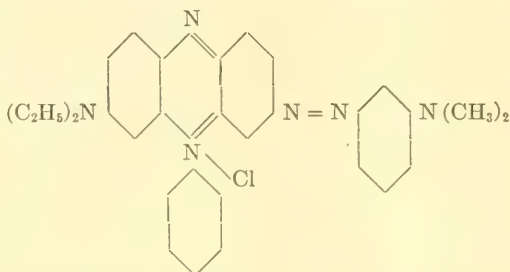


⁷ Diazingrün S(K) and Halbwohlgrün B(M) are also janus greens but it is not clear which of the following formulae they possess.

2. Janus green G(Farbwerke Hoechst Co.) dimethylsafraninazodimethylanilin chloride



3. Janus green B(Farbwerke Hoechst Co.) diethylsafraninazodimethylanilin chloride



Only the latter, janus green B, will stain mitochondria, though the others differ only in the substitution of an H_2 or $(CH_3)_2$ in the place of the $(C_2H_5)_2$ group. The poor results obtained with some samples of janus green are probably due to admixtures of the first and second varieties. The azodimethylanilin has but little to do with the specificity of the reaction because it is well known (Shipley '15, p. 86 and Cowdry '14, p. 269) that the diethylsafranin alone will stain the mitochondria more or less specifically. Moreover, I have prepared the safranin from the janus green of Grüber, the dimethylsafranin, from the janus green G of Hoechst, and the diethylsafranin, from the janus green B of the same firm, and I find that the diethylsafranin, alone, will stain the mitochondria.

There is, in addition to these janus greens, a large series of other janus dyes, manufactured by the Farbwerke Hoechst Company, which thus far have escaped the attention of his-

tologists. I have made a special study of them. Janus blue G and R, janus grey B and BB, janus black D, I, II and O and janus yellow G and R are of particular interest because they are safranin derivatives, the others being dyes of the triphenylmethane and other series.

Janus blue is diethylsafranin- β -naphthol and it stains mitochondria in living lymphocytes in a constant and specific fashion. It is inferior to janus green in that it will only stain mitochondria in these cells in a dilution of 1: 300,000. As an indicator of processes of reduction it is, however, better than janus green for the contrast between the blue of the dye itself, and its red safranin base is more brilliant than in the case of janus green. The marks 'G' and 'R' indicate, according to Schultz ('14, p. 48), that the janus blue is made by two processes, from clematin (mark 'G') and from safranin T (mark 'R').

Janus black I also stains mitochondria in living blood cells specifically, but, on analysis, I find that it is not a pure dye but a mixture of two substances, diethylsafranin-azodimethylanilin and a brown substance, the nature of which I am unable to determine. Thus, the specificity of janus black I for mitochondria is undoubtedly due to the fact that it contains janus green as one of its ingredients.

I have isolated the diethylsafranin from janus blue, janus black and janus grey (I failed with janus yellow) and they all stain mitochondria, which is further evidence that the specificity of janus green depends upon the diethylsafranin group. It may be said that the staining is favored by the addition of azodimethylanilin to it, as in janus green; increased, though not so much by adding β naphthol; and altogether prevented by the addition of other groups in janus grey.

It is exceedingly difficult to determine whether this staining of mitochondria is a chemical or a physical process. Chambers ('15) has arrived at the conclusion that it is not due to a chemical combination.

RELATION TO METABOLISM

There is ample evidence that mitochondria play an active and fundamental rôle in cell activity, though just what the part is, is obscure.

The first fact of interest is their wide occurrence. They occur in the majority of the cells of all animals which have been investigated, with adequate methods of technique, from man to the most lowly protozoan. Plants also contain them. They are most abundant in the active stages of the life of the cell. They diminish progressively in number as the cells become senile. The most striking example of this is seen in sections of the skin as one passes from the cells of the deeper layers, which contain many mitochondria, to the more superficial, desquamating cells, which are dead or dying and which often are quite devoid of mitochondria. Moreover, the mitochondria decrease in number as one passes from nucleated to nonnucleated red blood cells. Indeed, Shipley ('15, p. 83) has shown that the persistence of a few mitochondria in nonnucleated red blood cells is indicative of the fact that they are young and vigorous. It is well known that mitochondria are particularly abundant in immature, embryonic cells, which as yet exhibit no specialized activity, but in which metabolic processes are very active.

Direct experimental evidence is also at hand. There are many observations on quantitative variations in mitochondria with cell activity. Thus Romeis ('13, p. 12) has found that mitochondria are very numerous in actively regenerating tissues; Busacca ('15, p. 232) found that they decreased in number with fatigue in the cells of the retina stimulated with intense light; Homans ('15, p. 12) associated the number of the mitochondrial filaments with an increased activity of islet cells in experimental diabetes; Policard ('10, p. 284) showed that there was an increase in the number of mitochondria in kidney cells on administration of phloridzin, and so on.

These statements relate, however, only to the general impression given by the study of sections. There has been no attempt to distinguish, in a clear cut way, between absolute and relative

fluctuations in the mitochondrial content. The observations have not been controlled by a careful estimation of cell volumes. Thurlow ('16, p. 253) has been the first to realize these discrepancies. She has established a definite mitochondria cytoplasmic ratio in the nerve cell just as Hertwig years ago measured the nucleus cytoplasmic ratio.

There have been no carefully checked observations on qualitative changes in mitochondria with cell activity notwithstanding the fact that we have abundant evidence to show that the solubilities of mitochondria do vary. Of course the changes in form of mitochondria have been subjected to careful scrutiny, but so far they have yielded us little of value. The observations of Holmgren ('08, p. 308) on the changes in mitochondria in muscular fatigue are qualitative in a sense, and very interesting, but they have never been confirmed.

Relying on this rather meagre information investigators are generally inclined to believe that mitochondria participate in some of the processes involved in cell metabolism. Coghill ('15, p. 350) is rather more specific for he relates mitochondria to the constructive side of metabolism. Mayer Rathery and Schaeffer ('14, p. 619) attempt to narrow down the function of mitochondria still further. They claim that they take part in the processes of oxidation and reduction. This suggestion has also been tentatively advanced by the Lewises ('15, p. 393). It is in accord with the fact that mitochondria occur in all plants which have been examined, with the possible exception of some of the lower algae and bacteria, though I have even found granules staining specifically with janus green in some of the latter. It also falls in line with what we know of the janus green reaction.

RELATION TO HISTOGENESIS

The origin of the idea that mitochondria are concerned with histogenesis is not difficult to trace. They occur in all embryonic cells. In early stages of development they are the only formed elements in the cytoplasm. They are filamentous in the myoblasts and neuroblasts and it is perfectly natural to think that

they become transformed into fibrils and other products of differentiation, but the trouble is that the ways of nature are not simple, that the obvious interpretation is not necessarily the correct one.

In looking over the literature one is confronted with an appalling mass of conflicting observations, no unanimity is evident, as was seen in the work on the chemical constitution of mitochondria. Certain investigators have overstepped the mark. For example, mitochondria were seen and described in adult nerve cells by Altmann ('90, p. 52), Levi, ('96, p. 3), Nageotte ('09, p. 827) and others. Hoven in 1910 arrived at the conclusion (p. 475) that the mitochondria are transformed into neurofibrils in the developing nerve cell. This was generally accepted (Firket '11, p. 545 and Arnold '12, p. 289). Since it was supposed that mitochondria become transformed into neurofibrils it was natural to think that they were absent in the adult nerve cell after neurofibril formation has ceased. Meves ('10, p. 655), Hoven ('10, p. 478) and certain others expressed this opinion, and it fell to my lot to rediscover mitochondria in the nerve cell ('12, p. 497) and to show that the evidence is not conclusive that they become changed into neurofibrils ('14a, p. 416).

But critical mention need only be made of the claims that mitochondria transform into hemoglobin (Schridde '12, p. 517), pigment (Asvadourova '13, p. 293), collagenic fibrils (Meves⁸ '10, p. 150), zymogen granules of the pancreas (Hoven '10a, p. 350), goblet cell-mucus (Grynfeltt '13, p. 10) and to the suggestion of the same author that they also form the colloid substance of the thyroid gland ('12, p. 147).

The evidence is based chiefly upon similarities in form, which do not mean very much, and upon similarities in the staining reactions of fixed tissues, which mean still less. Moreover, hemoglobin, and the variety of pigment mentioned by Asvadourova, are both chromoproteins, one having the pyrrol nucleus and the other containing tyrosin; while collagenic fibrils

⁸ Meves' line of reasoning is interesting as well as unanswerable for he assumes that the mitochondria are invisible (not staining with either iron hematoxylin or fuchsin) when they form the fibrils (p. 164.)

on boiling yield gelatin, a protein devoid of tyrosin. One is tempted to enquire by what chemical changes can the mitochondrial substance, which is supposed to be a phospholipin combined with a small fraction of albumin, form both? There is no evidence that the mitochondria give a positive Adamkiewicz or Millon's reaction. It is, furthermore, difficult to conceive of how pancreatic zymogen, which is presumably the precursor of steapsin, amyllopsin and trypsinogen; mucin, which is a glycoprotein devoid of iodine; and the colloid of the thyroid, which contains iodine can originate from one and the same substance.

But the strongest evidence in favor of a change in mitochondria comes from the botanists, because mitochondria can be easily seen unstained in living plant cells and the substances, which are supposed to be formed from them, (xanthophyllous and anthocyanic pigments, chlorophyll, etc., Guillaumond '13, p. 436) are themselves naturally colored. Still it is apparent that the doctrine of an actual chemical transformation of mitochondria into substances of diverse constitution is weak.

Regaud ('11, p. 685), perceiving the difficulty, advanced his 'eclectosome' theory⁹ according to which the mitochondria play the part of plasts, choosing out and picking up materials from the cytoplasm and blood stream, condensing them and converting them, in their substance, into infinitely diverse products. Chemical substances are thus supposed to be drawn in from the outside, not to be formed through a transformation of mitochondrial substance. But Regaud goes too far in comparing the mitochondria to the side chains of Ehrlich, which are even now going out of fashion, and possibly also in endowing them with the ability to choose and select, for it is quite conceivable that they may play an entirely passive rôle in histogenesis. They may act only as a vehicle or substratum, in which, by virtue of its physical or chemical properties, substances are deposited which are synthesized through the activity of the cell as a whole.

⁹ This conception is a modification of the famous lipid membrane theory of Overton, the chief difference being that the lipid substance is said to be distributed throughout the whole area of the cytoplasm in the form of mitochondria, instead of being confined to a layer on the surface of the cell.

The problem is evidently not a simple one. We must weigh, with caution, assertions of the transformation of mitochondria into other chemical substances, appreciating well the little that is known of the chemistry of the processes involved. Mitochondria unquestionably are associated in some way with the formation of many substances: I have emphasized the improbability of their being transformed into them. Yet we must not follow the regular plan, go altogether too far in our reasoning and imagine that mitochondria never change, only it is likely in some cases, in others it is not. It would not require a great stretch of the imagination to conceive of mitochondria as being changed into lecithin (Bobeau '11, p. 393) or neutral fat (Dubreuil '13, p. 142), though Leathes ('10, p. 115) suggests that the reverse is true, that phospholipins are built up from neutral fat. One is naturally inclined to consider most seriously those studies (Chambers '15, Casteel '16) in which janus green is used with living material because some of the so-called mitochondrial methods are far from specific and are inclined to deceive the unwary.

RELATION TO INHERITANCE

Benda and Meves were the first to claim that the mitochondria may constitute, in part, the material basis of heredity. The claim is based on the well-known experiment of Godlewski which seemed to show that an egg, deprived of its nucleus, when fertilized with sperm of another species, retained certain maternal characters on development. In fact there is nothing novel in the conception that there is such a thing as a cytoplasmic heredity. Jenkinson ('14, p. 152) and Conklin ('15, p. 176) freely admit it. What is new is the view that mitochondria carry it. Van der Stricht ('09, p. 80) showed that the penetration of the sperm into the egg is total in the bat. Moreover, there are the positive observations of an ever increasing number of investigators that paternal mitochondrial substance enters the egg on fertilization. Space only permits of reference to Meves' demonstration of this in *ascaris* ('11, p. 709), Levi's in the bat ('15, p. 488) and of Duesberg's in *ciona* ('15, p. 41).

The adherents of the chromosome hypothesis in this country and elsewhere are naturally opposed to this view. It is said that the cases in which it has been shown that mitochondria pass into the egg on fertilization are exceptional and the crucial cases are those in which no mitochondrial substance passes into the egg. This Lillie believes to be the case in nereis. Mitochondria generally occur in the middle piece and tail of the spermatozoon, though this is not always true. Lillie ('12, p. 418) says that "the middle piece and tail of the spermatozoon do not enter in the fertilization of *Nereis*." He admits (p. 426) that "it is possible that the fixation granules introduced by the spermatozoon represent a cytoplasmic element." So that, until new facts are discovered, through the use of mitochondrial methods of technique, the case of nereis does not offer an insurmountable barrier to the acceptance of the view that mitochondria play a part in inheritance. Notice, the claim is not made that mitochondria constitute the sole material basis of heredity, because even though there be a cytoplasmic heredity in some forms it does not follow that it is universal, quite apart from the fact that such a statement would be absurd. It is interesting to observe that Wilson ('14, p. 352) has recently admitted the possibility that mitochondria may function in heredity.

To my mind the greatest obstacle in the acceptance of the view propounded by Benda and Meves is our suspicion of the chemical nature of mitochondria. If it is true that they are phospholipins it is hard to regard them as carriers of heredity even though they contain albumin also. It cannot be denied that, chemically, chromatin appears to be best fitted to play the part of heredity carrier. Even, should it be shown that mitochondrial substance passes over in fertilization in all animals it may indicate nothing more than that a living portion of the sperm, capable of metabolism, enters the egg. It is a mistake, however, to arrive at a hasty conclusion because those who make the conservative statement that mitochondria play some part in heredity occupy just as secure a position as those, on the other hand, who claim that chromatin is the sole heredity carrier.

PATHOLOGY

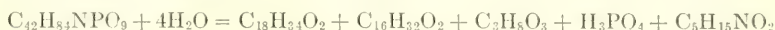
The statement of Beckton ('09, p. 191) that mitochondria are absent in malignant growths, as contrasted with benign tumors was instrumental in kindling interest in the study of mitochondria in tumor cells, in spite of the fact that the assertion was false, as Bensley ('10) discovered later. One is tempted to entertain, as a working hypothesis, the view that mitochondria may serve, in a measure, as indicators of the effect of X-ray, radium and other therapeutic agents on tumor cells; for we know that they respond very readily to injury, and, since their chemical make up is entirely different from nuclear chromatin, they may serve as clues to a different type of activity. But unhappily the problem has attracted so little attention that we have not even an accurate and comprehensive account of the relations of mitochondria in tumor cells, and of the effect of X ray and radium on normal cells to begin with (Beckton and Russ' observations on radium need confirmation). Besides this, tumors offer a new and attractive field for the study of the behavior of mitochondria in histogenesis. Nobody has even touched on the relation of the myofibrils, in myomata; and of the connective tissue fibrils, in fibromata; to mitochondria.

By virtue of the little that we do know of the function and chemical constitution of mitochondria we naturally seek for information about them in certain types of pathological change.

The fatty degenerations and infiltrations are of particular interest. Mayer, Rathery and Schaeffer ('14, p. 608) and Scott in some as yet unpublished work on the pancreas found that the mitochondria agglutinate and undergo definite alterations and he traced their relation to the genesis of the fat-like droplets within the cells in experimental phosphorus poisoning. Moreover, since they are soluble in chloroform and other anaesthetics, it is altogether likely that they may show interesting changes in narcosis. And the well-known fact that certain unknown fat-like substances are the point of action of tetanus toxin in the nervous system lights the way to the study of mitochondria in tetanus.

The single observations of Policard ('12, p. 229) on the temperature solubility of mitochondria are, perhaps, not without significance. He found that exposure to a temperature of from 47–50°C. for 30 minutes dissolved the mitochondria in kidney cells without affecting the appearance of the nuclei. It is well within the bounds of possibility that a prolonged or intermittent temperature of say 41°C. (105.8°F.), as in a high fever, may bring about a solution or chemical alteration of mitochondria in some or other cells of the body. This is, at any rate, an interesting thought in connection with Welch's ('88, p. 403) belief that fatty degeneration of the heart muscle is in some way associated with high fevers for we have evidence that mitochondria are chemically related to the phospholipins.

Two interesting hypotheses have been advanced on the supposition that mitochondria liberate cholin, which is quite likely since on hydrolytic dissociation lecithin, a typical phospholipin, yields cholin (Mathews '15, p. 91):



Macklin ('16) has found that they are particularly abundant in the constriction between the two parts of nuclei dividing by amitosis and advances the view that they may be giving off cholin, which, if Robertson's supposition is correct, may lower the surface tension locally and thus facilitate the nuclear constriction. Cowdry ('16) has made the suggestion that cholin may be set free in the nervous system through the disintegration of mitochondria, and that, inasmuch as organic diseases of the nervous system can be separated from functional neuroses by the formation of cholin in the one and not in the other (Halliburton '07, p. 74), it is possible that a study of mitochondria may afford a cytological basis of distinction between these two groups of nervous diseases.

Thus far no account of mitochondria in acidosis has appeared. Now it is common knowledge that mitochondria are very sensitive to acids. It is also well known that one of the first manifestations of acidosis is a marked inhibition of the respiratory oxidation of the cell (Mathews' 15, p. 247). If there is any-

thing in the theory that mitochondria function in processes of oxidation and reduction it is possible that these two facts may be related. Let us remember also the dyspnoea in acidosis. Moreover, mitochondria respond to a wide range of noxious influences by swelling up before going into solution, which might be due to the effect of increased H ion concentration¹⁰ upon their protein fraction causing it to become hygroscopic and to swell. The affinity of injured cells for basic anilin dyes is probably due to a swing of the reaction in them toward the acid side.

The path is now ready for the study of mitochondria in pathology because Ciaccio ('13), and others, have cleared the way by their careful descriptions of the post-mortem disintegration of mitochondria. It is true that mitochondria are so delicate that they disappear in the pancreas very soon after death, but the same difficulty is not met with in the case of the nervous system and other organs which autolyse more slowly. Key has found mitochondria in spinal ganglion cells 24 hours after death. It is no longer necessary to employ the poorly penetrating osmic acid containing fixatives because a simple mixture of formalin and bichromate, as advised by Regaud, will, with slight modifications (Cowdry '16) answer the purpose much better. While in some cases the usual fixation in formalin, followed by mordanting in bichromate, will fix the mitochondria satisfactorily (i.e., in human brains).

TECHNIQUE

Janus green, diethylsafranin and janus blue are the most useful vital stains for mitochondria. They should be applied by injection through the blood vessels in a dilution of about 1: 20,000 of physiological saline. Mitochondria may also be stained by simply immersing the tissue in the dye, but the objection to doing this is that the dyes penetrate very slowly indeed, so that only the most superficial cells are stained and often very unevenly at that. Special difficulties are often met with in invertebrates because janus green is only slightly soluble in sea water, and because it precipitates and stains intensely

¹⁰ As suggested to me by Dr. R. R. Bensley.

certain proteins in the body fluids. Its toxicity varies in different cells. Cowdry ('14, p. 279) observed mitochondria stained with janus green in neutrophile leucocytes during amoeboid movement and phagocytosis of minute foreign particles, and Shipley ('16) also studied mitochondria vitally stained with it in actively motile trypanosomes.

Mitochondria are easily fixed, osmic acid and the mixtures of Altmann ('90, p. 27), Benda ('01, p. 163), Meves ('08, p. 832) and Bensley ('11, p. 308) are used frequently. They all contain osmic acid. Personally, I have virtually abandoned them because they penetrate so badly. The simple mixtures of formalin and bichromate (Regaud '10, p. 296) work much better, especially if the formic acid is neutralized with magnesium carbonate, on account of the sensitivity of mitochondria to acids. Moreover, formalin and bichromate often give good fixation in tough fibrous tumors and in eggs filled with yolk, which are hard to fix in the older fixatives.

Generally speaking, the mitochondria may be stained by all methods irrespective of the way they have been fixed. The Altmann method and its modifications (Bensley '11, p. 309; Cowdry '16, and others), the Benda ('01, p. 163) method, and iron hematoxylin are most used, though Bensley ('16, p. 47) has just devised a new technique by which the mitochondria are stained with Brazilin. The fuchsin methyl green modification of Altmann's method gives the most brilliant results, but fades after a year or two. The Benda method is much more permanent. Iron hematoxylin is the most lasting of all, but it is rather less specific. Remarkably good results may be obtained with it after formalin bichromate fixation. Since the hematoxylin is very closely bound to the mitochondria a variety of useful counterstains may be used. Thus, toluidin blue or pyronin, or indeed any basic dye, will stain the Nissl bodies in nerve cells beautifully, the mitochondria remaining black. Moreover, sections of the pancreas of the mouse counterstained with safranin and light green will often show the blebs on the mitochondria (which are by some supposed to be forerunners of zymogen) stained red against a green background, the mitochondria and zymogen granules of course being blue-black.

SUMMARY

One is assailed with the feeling that we know very little about mitochondria. This it would be foolish to deny. Therein lies their charm. One also has the uncomfortable impression that it is easy to prove that mitochondria can do anything by searching the literature diligently for the required statements and ignoring the rest. A great deal of work will have to be gone over, confirmed and corrected. Certain it is, however, that we can at least define them in microchemical terms as well as in a morphological way. As far as breadth of distribution goes they yield place to no other type of cell granulation. They are as characteristic of the cytoplasm as chromatin is of the nucleus. We know, further, that they are delicate indicators of cell activity and we suspect many things.

This line of study is to be considered as a revival of interest in protoplasm. It may well be asked why we have not heard of mitochondria long ere this? The reason is not far to seek. It is the direct outcome of the tendency to neglect the cytoplasm and to concentrate attention upon the nucleus on account of the interest which seems to center in the nucleus from the standpoint of heredity. The aim in making up fixatives was to show nuclear detail. For this purpose mixtures containing either alcohol, chloroform or acetic acid were employed because of their rapid penetration. Now these substances destroy mitochondria. So that the more attention was focussed on the nucleus the less chance there was for the observation of mitochondria. A vicious cycle was maintained. Happily a reaction is now taking place, an equilibrium is being produced.

This line of study has also developed parallel with the recent tendency among physiological chemists and pathologists to become interested in the phospholipins, which are complex compounds of fatty acid, phosphorus and nitrogen and among which we are inclined to group the mitochondria. Whereas, formerly, their whole attention was devoted to the study of proteins and was dominated by the tremendous impetus of Emil Fischer's work on protein synthesis, based as it was upon

Kossel's theory of the nature of the protein molecule, which attracted world wide notice because of the psychological factor involved in the supposed manufacture of living substance.

It is impossible to predict whither this mitochondrial work will lead us in pathology, certainly, however, toward a truer appreciation of the importance of the behavior of protoplasm in pathological conditions, because now we have a cytoplasmic criterion of cell activity as well as a nuclear one.¹¹

It has already proved fruitful in connection with our conceptions of cell structure, for it has enabled us to advance one step further than Schultze did when he defined protoplasm as being a glass-like, semifluid material in which granules are embedded. We can now recognize, with precision, among his granules one great class, the mitochondria, which are more or less distinct chemically and morphologically and which we have good reason to believe occur in almost all protoplasm. The movements of mitochondria in living cells tend to confirm our deeply rooted mistrust of the ancient doctrine of a cytoplasmic reticulum, which, as I have already said persists in the form of misleading diagrams in even our most modern text books of histology. Moreover, this line of investigation has placed us in a position where we can consider in a new light certain conceptions of the constitution of protoplasm, Altmann's 'bioblast' and Flemming's 'filar' theories in particular, for we recognize in some of the 'bioblasts' and in some of the 'fila' our mitochondria. We appreciate their superficiality and we realize as never before that we will have to look far deeper for clues as to the nature of that mysterious and remarkable organization in living matter of which the phenomena of polarity and bilaterality are the visible expressions.

¹¹ Very recently Goetsch has demonstrated, in a paper published in the May number of the Johns Hopkins Hospital Bulletin, that an increase in mitochondria is associated with an increase in the activity of the thyroid epithelium and with the severity of the clinical symptoms of hyperthyroidism.

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THE EMBRYOLOGY OF THE BIRD'S LUNG¹

BASED ON OBSERVATIONS OF THE DOMESTIC FOWL

PART 1²

WILLIAM A. LOCY AND OLOF LARSELL

FORTY-SIX FIGURES

FOREWORD

The substance of this paper is the result of a combination of observations on the part of the senior author extending over several years, and the recent observations of Mr. Larsell who came to the laboratory in 1913 as Fellow in Zoölogy. It was agreed at the outset to review work already done, to extend it and to collaborate in the publication of a paper on the development of the avian lung. While, as to design, the investigation originated with the senior author, as to execution, the observation has been unequally divided and it is only fair to say that the details of the development of the bronchial tree, including the air-sacs, and of the recurrent bronchi, are the result of the investigations of Mr. Larsell. The narrative and other embryological observations have fallen chiefly to the senior author.

We have had the use of drawings and of manuscript theses of Mr. G. H. A. Reeh and of Miss Mary Head, former graduate students working in the laboratory. The use of their sketches has been helpful, but no personal observations have been borrowed. Fifteen sketches by Mr. Reeh, retouched and corrected, have been reproduced in the body of the text.

W. A. L.

¹ Contribution from the Zoölogical Laboratory of Northwestern University, William A. Locy, Director.

² Part II of this paper will appear in the July issue.

It is commonly recognized by morphologists that our knowledge of the development of the avian lung including its air-sacs is both inadequate and defective in several important respects. The notable observations of Schulze ('11) and of Juillet ('12) have brought forward a newly recognized structural element, the recurrent bronchi, known only in the lung of birds, and which imparts a renewed interest in the structural peculiarities of the avian lung and the physiology of its air-sacs. It is now more imperative than heretofore that we should have a review of the embryological history of the lung with a more precise study of the development of the bronchial tree, of the air-sacs and their recurrent bronchi.

The assumption that, except for air-sacs, the lungs of birds and of mammals are essentially similar as to architecture has retarded the recognition of the structural peculiarities of the bird's lung. The beginnings are similar in these two classes of vertebrates but the end-products are very different. There is needed an embryological study to determine the way in which the avian lung departs from the mammalian type and to determine the precise nature of the intercommunications between its bronchioles. The development of recurrent bronchi from the air-sacs and the establishment of labyrinthine communications between all parts of the bronchial tree, imparts to the avian lung a unique architecture not found in any other class of vertebrates. There is no ending of the ultimate twigs of the bronchial branches in culs-de-sac, as in the lungs of other vertebrates, so that the facilities for ventilation of the avian lung are very complete. The absence of alveoli in which a portion of the air is retained as residual air, permits the air current to sweep unimpeded through the minutest air passages and affords great opportunities for respiratory exchanges between the blood capillaries and the air capillaries. The air-sacs receive their supply through direct orifices, during inspiration, and the air passes from these reservoirs into the lung by way of the recurrent bronchi during expiration. It is essential to understand the intercommunications of the air passages in order to comprehend either the morphology or the physiology of the bird lung.

In early stages of development the outgrowths of the bronchial tree end blindly and this condition is maintained in the adult mammalian lung, but in the bird lung, the terminals come into contact and anastomose during embryonic development so that in the adult lung there are no culs-de-sac. This condition of anastomosis affects also the air capillaries that are radially arranged around the parabronchi. Thus in following the development of the air passages of the bird's lung we pass from the primary condition of a bronchial tree to the modified condition of uninterrupted bronchial circuits.

Some of the points that require elucidation for understanding the morphology and the physiological action of the avian lung may be enumerated:

As a background, a knowledge of the phenomena of extra pulmonary development, or the general course of its embryology.

The intra-pulmonary development of the bronchial tree, its ramifications and the establishment by anastomoses of unbroken communications between the parabronchi and the air capillaries.

To determine the method of formation of the air-sacs and of their outgrowths, the recurrent bronchi.

To observe the formation of the air capillaries and the establishment of anastomoses among them.

To observe the origin and mode of development of the pulmonary artery and of the general course of circulation within the lung.

In addition to the above there should be observations on the diaphragmatic membranes and the muscular means of producing respiratory movements accompanied by physiological experiments, but observations of this nature have not been included in our studies.

Our observations are confined to the embryology and morphology of the lung and air-sacs, and in this study of limited range we do not presume to have found answers to the ultimate questions of morphology of the bird lung. We have assembled our results merely as an objective account of what we have been able to observe in the time and with the material at our disposal.

The observations are brought under consideration in the following order:

1. The external aspects of lung development.
2. The development of the bronchial tree.
3. The air-sacs and the recurrent bronchi.
4. The development of the pulmonary artery.

Followed by comments on the steps of progress in the anatomical analysis of the bird's lung and comparison of some of our results with those of previous observers.

Comments on the literature. In dealing with the extensive literature of the avian lung one is confronted with the dilemma of choosing between a comprehensive chronological mention of the observations of the different investigators or a very condensed selective review of the results of a few workers. The latter plan on the whole seems better, since the literature has been repeatedly reviewed (as in Flint's contribution, '06, in Juillet's, '12, and in the papers of others); moreover, genuine advances are contained in a limited number of papers.

As to embryological observations, the chief contributions are by Rathke, '28; Von Baer, '28; Remak, '55; containing the first figures of the buds of the ecto- and entobronchi; Selenka, '66, on development of the air-sacs; His, '68, laryngo-tracheal groove and trachea; Zünstein, '00, bronchial tree and air-sacs; Moser, '02, method of growth; Bertilli, '05, air-sacs; Juillet, '12, comprehensive treatise; besides text-books, as Foster and Balfour '74; Marshall, '93; Lillie, '08, etc.

As to intra-pulmonary anatomy of adult stages: Sappey, '47; the bronchial passages especially analyzed by Campana '75; Huxley, '83 bringing the terms mesobronchium, ecto, ento, and parabronchia into common use; F. E. Schulze, '09, '10, '11, bronchial tree and air-sacs; Miller '93, comparative structure of lungs including birds; Guido Fischer, '05; Juillet, '12.

Histology: F. E. Schulze, '71; Oppel, '05.

The air-sacs have been extensively described in the adult without involving the anatomy of the lungs as by Guillot, '75, comparative; Bruno Müller, '07, pigeon; Schulze, '10, etc.

As to methods of growth: Aeby, '80, monopodial; Miller, '93, comparative, budding predominates in birds, septum formation secondary; Moser, '02, budding the uniform principle of growth in birds and other vertebrates; Flint, '06, paper on mammals but reviews the literature on other vertebrates and comments on the method of growth in birds.

Campana's thorough and extensive paper of 1875 requires separate mention. It is part of a general plan designed to illustrate the laws of genesis and evolution, and the primary title of his memoir is *Physiology and Respiration of Birds*. Nevertheless, the anatomical part is of chief importance, and it is the most critical and comprehensive treatise on the structure of the adult bird's lung to which we have had access. This memoir is not easily accessible, and although it is commonly mentioned in the literature lists, it has, unfortunately, been little read. Campana makes an illuminating analysis of the bronchial passages, tracing their ramifications in detail and making an especial point of the bronchial circuits which unite the various divisions of the bronchi into a plexus of intercommunicating passages. He also noticed the recurrent bronchi but without understanding their significance. Further mention of this point will be made later, and, also, his classification of bronchi will be explained in our section on the bronchial tree.

F. E. Schulze in 1911 published an important paper on the comparative anatomy of the air-sacs in the adult and for the first time ('09) described the recurrent bronchi and pointed out their physiological office. His excellent methods of injection with metal and celloidin are described in detail.

The most recent important contribution to the morphology of the bird's lung is the paper of Juillet published in 1912. This is a comprehensive treatise embracing an anatomical, embryological, histological and comparative study of the avian lung. It contains a review of previous work and a list of the literature. Its most significant feature is the description of recurrent bronchi (discovered by Schulze, '09 and '11) growing from the air-sacs into the lungs and anastomosing with the parabronchi of ecto, ento and laterobronchi. He used metallic injections of

Wood's metal and Darcet's metal besides plastic reconstructions and the usual embryological and histological methods. An adequate review of this excellent paper would require much space and it should be read in the original. Our observations differ in some particulars from those of Juillet (especially as regards the origin of the interclavicular air-sac) and these differences will be commented on later.

Technique. Chick embryos from the close of the second day up to the time of hatching were used in observations on the developing lung. The stages were compared with the figures in Duval's *Atlas d'Embryologie* and his chronology adopted.

For dissecting, fresh embryos were first immersed in a solution of 8 per cent formalin and preserved in a 4 per cent solution. While the heart was still pulsating a large number of the embryos for dissection were injected with India ink through the vascular area or the liver according to the age of the embryo. Dissections were made of stages from three days to hatching, of young chicks one, two and three days after hatching and of adults.

For imbedding, the embryos were fixed in Kleinenberg's picrosulphuric solution and in formalin. Stages from 48 to 96 hours were sectioned from eight to ten microns in thickness and sections were also made of older stages and of the lung parenchyma of the adult.

It would have been impossible to work out with any degree of satisfaction the development of the bronchial tree and of the recurrent bronchi without the use of a method originated by Hochstetter (*Zeitschr. für Wissenschft, Mikr. und Mikr. Tech.*, Bd. XV, '98) of using clove oil and chloroform. This method was modified by using rather thick cedar oil instead of clove oil which was found to give clearer preparations and those of longer duration.

In stages subsequent to 96 hours, the lungs and air-sacs were dissected out of the previously fixed and hardened specimens, then cleared in cedar oil, after which the organs were placed in a mixture of one part cedar oil and two parts chloroform. On becoming permeated with this fluid, the preparation was removed from the mixture and placed on a filter paper until the

chloroform might evaporate. The evaporation of the chloroform served to draw the cedar oil from the lumina of the various branches of the bronchial tree into the lung tissue and to fill the spaces thus made with air. When this preparation was replaced in pure cedar oil, the difference between the refractive index of the imprisoned air and the surrounding medium gave the lung tubes the appearance of being filled with a metallic cast. Thus the minute air passages that could not be injected by other means were made clear. The finer details would disappear after a few minutes as the cedar oil percolated into them, but the same specimen, if carefully manipulated can be treated repeatedly without apparent injury, and a complete picture could finally be obtained. This method was successfully used in tracing the development of the bronchial tree up to the eighteenth day of incubation.

For later stages, celloidin and Wood's metal injections afforded the most helpful preparations. Such injections were also attempted of earlier stages, but the uncertainty of successful preparations and the destruction of the specimen employed made the air injections more satisfactory. This was especially true since the air injections showed fine points of detail that were not revealed by the more limited penetration of the fluid celloidin and the heated Wood's metal. Several preparations of the adult lung were made with the Wood's metal injections. Preceding the Wood's metal casts, the lungs of the freshly killed fowl were distended under pressure with 80 per cent alcohol until the air-sacs were fully expanded, after which the entire bird was immersed in alcohol for twenty-four hours or more before attempting metallic injection.

For histological study of the air capillaries of the adult bird, the pulmonary apparatus was injected under pressure with corrosive acetic fluid and by this means the lungs were fixed in a distended condition and the air capillaries were not collapsed.

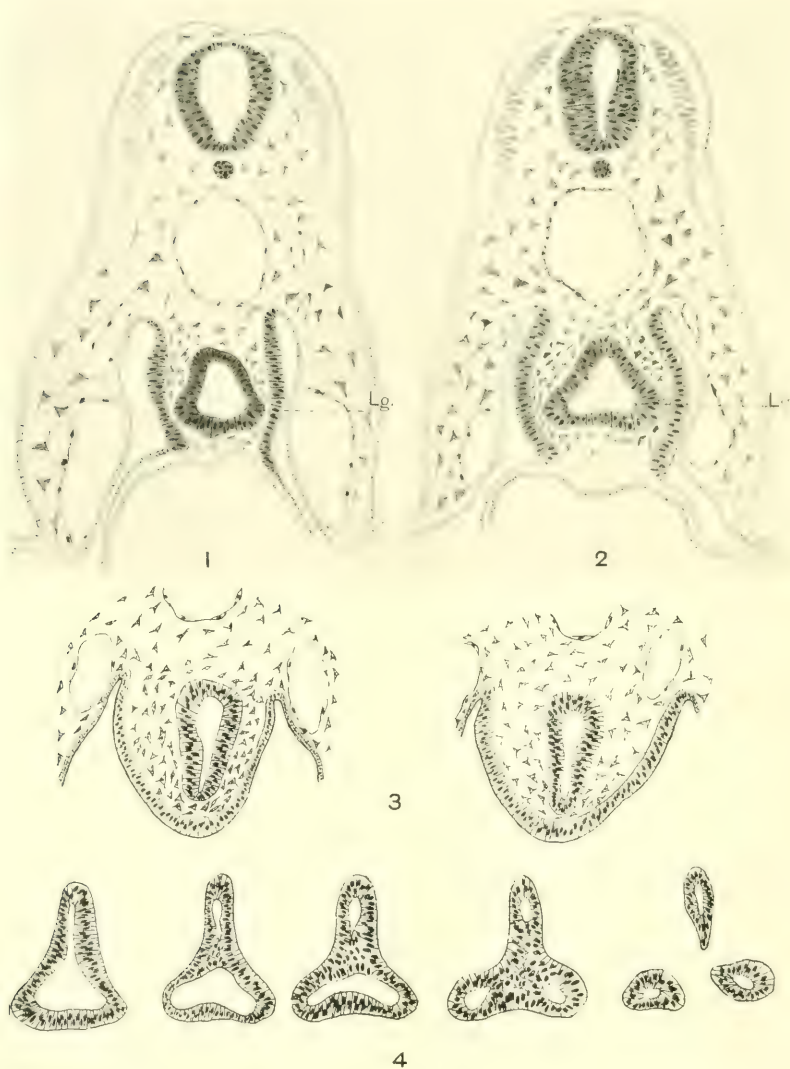


Fig. 1 Cross section through pharynx and lung pouches of a chick embryo incubated 51 to 52 hours.

Fig. 2 Similar section of a slightly older embryo (52 to 53 hours) showing the well defined lung pouches. Figures 1 and 2 drawn by Gilbert H. S. Rech.

Fig. 3 Two consecutive sections through the pharyngo-tracheal groove of the same embryo. These sections are respectively 120 and 128 microns in front of the one sketched in figure 2.

Fig. 4 Sections through the same region of an embryo incubated 55 to 56 hours.

1. THE EXTERNAL ASPECTS OF LUNG DEVELOPMENT

Under this heading the external features of lung formation will be described while the intra-pulmonary changes will receive separate consideration in the following section.

The time, the place and the method of formation of the primitive lung of the chick has been well described by various observers. In reference to the time, it should be remembered that in all embryonic development there is individual variation as well as variable methods of estimating stages. It is not, however, so important to establish an exact correspondence in chronology of different observers as to determine the method of lung formation and the normal sequence of changes.

The first external appearance of the lung of the embryo chick comes in the early part of the third day. Many specimens of 30-31 somites show a slight ridge-like enlargement on each side of the latero-ventral surface of the pharynx just behind the fourth gill-pouch. This is in the narrowed respiratory division of the pharynx, as distinguished from the broadly expanded branchial division.

Cross sections show that the ridge-like formation is owing to an evagination of endoderm into the surrounding mesenchyma. Figure 1, from a specimen of 30 somites, estimated as 50 hours' development, is cut through the more prominent part of this outgrowth. Figure 2 is from a slightly older specimen, estimated as the 52-hour stage. Both sketches are from camera lucida tracings, so that the outlines are correctly represented, but in finishing, the details, especially the nuclei of cells, have been made diagrammatic. The shallow pockets on the ventral border of the pharynx are the beginnings of lung pouches; they push out into the mesenchyma which is bordered by a very pronounced mesothelium. At their beginning, therefore, the primitive lungs are paired, and consist of two shallow pouches that open widely into the floor of the pharynx. The surrounding mesoderm is also a part of the lung anlage and increases *pari passu* with the growth of the endodermal part. The endoderm by budding gives rise to the lining membrane of the bronchial tree, the mesoderm

providing material for blood vessels, lymph spaces, muscles, connective tissue and like elements, while ingrowths from the ectoderm provide the nerve supply.

In front of the bulges the walls of the pharynx are compressed laterally and the tube is narrowed on its ventral border to form the laryngo-tracheal groove which is the forerunner of trachea and larynx.

Figure 3, showing the pharynx and the ventral groove, is a camera tracing of two consecutive sections taken 120 and 128 microns in front of the one sketched in figure 2. In still earlier stages the narrowing of the respiratory portion of the pharynx is easily seen as well as the incipient stages of the bulges from which the lung pockets are produced.

The cavity of the pharynx is also narrowed just above the lung pouches (fig. 4) so that in cross section, the outline is similar to that of figure 3 in an inverted position.

Immediately the lung pouches begin to elongate by growth of the endodermal lining in a caudo-lateral and somewhat dorsal direction, and prior to the 60th hour, their divergent distal ends become separated from the oesophagus (fig. 4). By the end of the third day (72 hours) the embryonic lung can readily be exposed by dissection.

At the close of the fourth day (96-hour stage) the lungs and adjacent territory present the appearance shown in figure 5. At this stage the lungs are small, smooth pouches extending caudally and dorsally along each side of the oesophagus. In this specimen, the sixth arch, from which the proximal end of the pulmonary artery is shortly to develop, has not been completed although a ventral spur of the arch is shown and a shorter dorsal spur from the aorta.

Figure 6 represents a ventral view and figure 7 a lateral view of the lung of a chick embryo near the close of the fourth day of development. The lung pouches are divergent and their distal portions extend caudad, laterad and dorsad. Their cavities are lined by the endodermal diverticula from the pharynx, and these are surrounded by mesoderm, so that the surface exposed by dissection is mesodermic. The walls of the endodermal tube

do not as yet show any buds (fig. 24). Although the differentiation of the trachea has begun it is not visible from surface views. Seen from the lateral aspect as in figure 7, the lung pouches, closely united with the walls of the oesophagus, pass below it and unite with the laryngo-tracheal ridge on the ventral

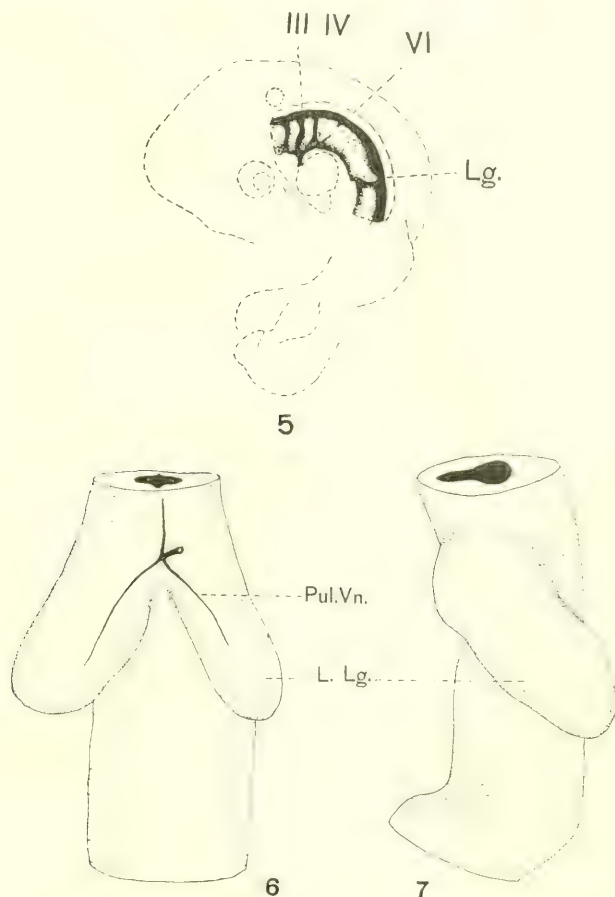


Fig. 5 Dissection of an ink injected chick embryo of 96 hours incubation exposing aortic arches and the left lung. Drawn by G. H. A. Rech.

Figs. 6 and 7 Surface views of the lungs of a chick embryo at the close of the fourth day of development. Figure 6 from the ventral and figure 7 from the lateral aspect.

part of the pharynx. The lung pouches are smooth and do not as yet exhibit surface irregularities.

Injected specimens of this stage frequently show blood vessels running along the ventral surface of each lung and uniting in the median plane at a point where the lung pouches join the pharynx (fig. 6). From this place of union a vessel leads into the left atrium of the heart. Another vein, coming from the front, passes along the ventral surface of the laryngo-tracheal groove and joins the stem vessel that leads into the left atrium. The blood vessels on the lungs are the beginnings of pulmonary veins and they are commonly injected before the pulmonary artery is established. Sections show however that vascular spaces for the formation of the distal extremity of the pulmonary artery are already present in the lung walls.

In the closing hours of the fourth day the trachea becomes differentiated from the posterior portion of the laryngo-tracheal groove. It may be definitely distinguished in an embryo with 39 somites (estimated as in the 94-hour stage), and, by the 100th hour, it is well defined. This is not readily evident in surface views but in optical section (fig. 25, 4 days, 4 hours) the connection of the trachea with the pharynx and with the bronchi is well exhibited. At the distal end of the lung tube is an enlargement that foreshadows the abdominal air-sac. At its proximal end, on each side, a short portion of the bronchus lies between the anterior limits of the lung and is the first appearance of the extra-pulmonary bronchus. These extra-pulmonary bronchi join the trachea which is of larger calibre than the bronchi. The oesophagus makes a rather abrupt dorsal bend away from the trachea, and then, with a more gentle curvature continues caudally and, bending downward, passes between the lungs.

During the fifth day the lung grows larger and begins to show surface irregularities. Figure 8 shows the appearance of the left lung territory as exposed by dissection in a specimen of the 4½-day stage. The lung pouch of this specimen has grown dorsally so as to extend across the path of the aorta. Its distal extremity exhibits a protuberance which is the beginning of a lobe in which lies the expanded end of the mesobronchus. At about this

stage the pulmonary artery is usually established and, in injected specimens forms one of the external anatomical landmarks. In the subsequent descriptions the pulmonary artery will be included as a feature of external anatomy but the details of its formation are separately considered under another heading.

In the specimen sketched in figure 8, the proximal end of the pulmonary artery shows as a spur from the sixth aortic arch, but owing to imperfect injection, the distal part that is formed in the lung wall is not seen. The rudimentary fifth arch is present in this specimen as a short vessel arising from the truncus arteriosus and joining the lower half of the sixth arch. Much variation exists as to the presence or absence of the rudimentary fifth arch and as to its dimensions when present. The degree of development of the pulmonary artery also varies in different specimens of this age.

Figure 9 represents a side view, and figure 10 a ventral view of the lung in the last half of the fifth day of development. The lobe at the posterior end of the lung pouch is shown in figure 9 and the trunk of the pulmonary is fully established. When viewed from the ventral surface (fig. 10) the right lung forms a somewhat greater angle (fig. 6) with the oesophagus than the left, producing an appearance of asymmetry. This asymmetry, however, is not owing to a difference in size of the lung (as in mammals and some reptiles) but to the pressure of the stomach (ventriculus) enlargement which begins at about this period. The greatest asymmetry comes about the middle of the fifth day; it is gradually rectified with the change in relative position of the viscera and the symmetry is restored by the eighth day. In figure 10 the pulmonary veins and the laryngo-tracheal branch are also shown on the ventral surface of specimen.

Near the close of the fifth day of development well injected specimens (fig. 11) show a network of blood vessels near the surface occupying a small area on the anterior dorsal part of the lungs. Sections and transparencies of this stage show that the network of vessels within the lungs occupies chiefly the dorsal region and is more extensive than appears from the surface. This figure shows also a branch from the pulmonary artery ex-

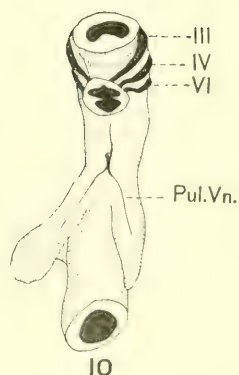
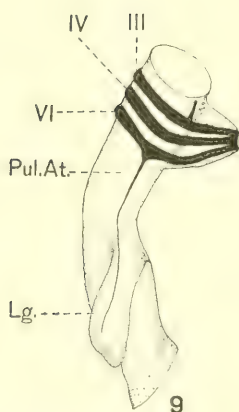
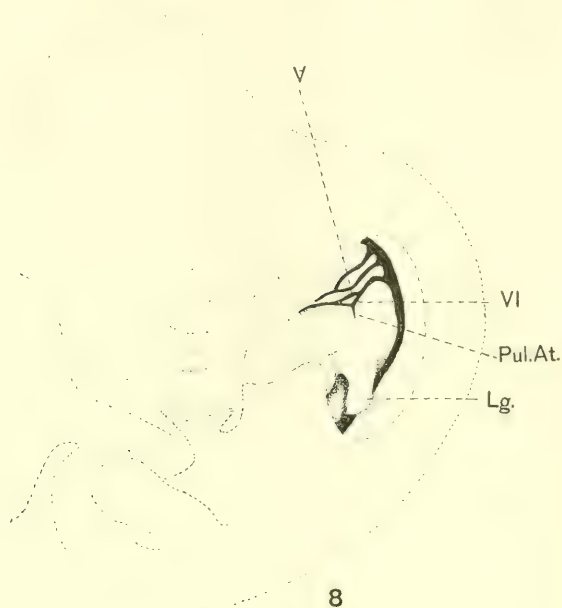


Fig. 8 Dissection exposing the lung and adjacent territory in a specimen of $4\frac{1}{2}$ days development (114 hours). Drawn by G. H. A. Rech.

Fig. 9 Side view of a dissection of the lung territory during the last half of the fifth day of incubation.

Fig. 10 Ventral aspect of the same specimen illustrating the apparent asymmetry of the lungs.

tending towards the trachea and passing through a network of capillaries which communicate with the vein, mentioned above, as running on the ventral surface of the laryngo-tracheal groove.

The immediately following external features of development may be rapidly passed over since, for some time, there is no significant change in the external appearance of the lung.

Figure 12 shows a dissection of the lung territory in an embryo of $5\frac{1}{2}$ days incubation and figure 13 a similar dissection of an embryo during the last half of the sixth day. In both these figures the anterior dorsal (cephalic) part of the lung is protuberant and the hook-like process at the caudal extremity is more evident than in earlier stages. They both exhibit the course of the pulmonary artery and pulmonary vein as seen in surface views, and figure 12 also shows in addition, a short ventral spur from the pulmonary artery.

A more comprehensive view of the superficial blood vessels of the lung is shown in figure 14, sketched from a specimen in the early part of the seventh day. The shape of the lung and the external appearance of both pulmonary artery and pulmonary vein are well shown. Especially to be noted is the trunk of the vein on its way to enter the left atrium of the heart, and the juncture with this trunk of the pulmonary vein and of the vein (laryngo-tracheal) running along the ventral surface of the trachea. A short arterial branch leaves the pulmonary artery on its ventral border in front of the lung and, passing through a capillary network, connects with the laryngo-tracheal vein. The anterior part of the lung above the pulmonary artery shows a superficial network of blood capillaries.

Although there is relatively little change in the surface appearance on the sixth day, it is to be understood that internal changes of great significance are taking place. The first branches of the bronchial tree arise on the sixth day of development and the network of internal capillaries is moulded over them. These internal changes are described in later divisions of this paper.

The seventh day stages, as seen from the side, show the lung approximating a rectangular outline with a protuberance from

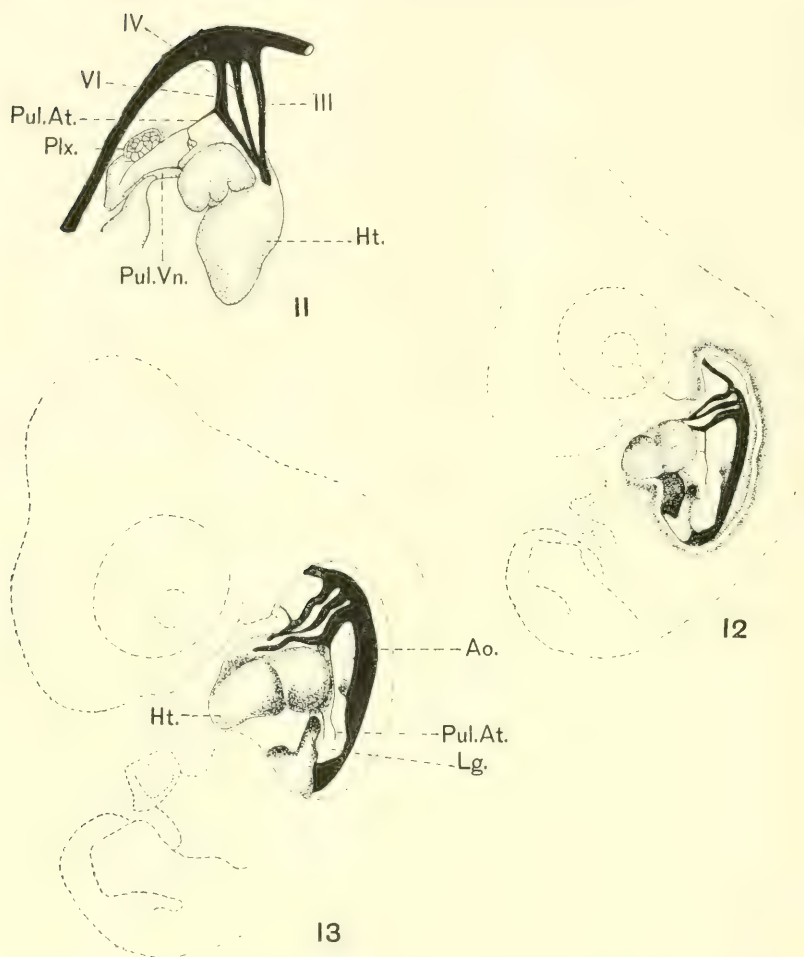


Fig. 11 Dissection of the lung territory of an injected specimen near the close of the fifth day of development, showing network of blood vessels on the anterior dorsal area of the lung, also the opening of the pulmonary vein into the left atrium.

Fig. 12 Dissection of the left side of an injected embryo of 5½ days incubation. Increase in size of lung and of pulmonary artery is evident. Projecting ventrally from the pulmonary artery just above the heart is a small blood vessel which anastomoses through a capillary network with the laryngo-tracheal vein as shown in figure 14. Modified from a sketch by G. H. A. Rech.

Fig. 13 Dissection of the left side of an injected embryo of the last half of the sixth day of development. Projections on the lung, pulmonary artery and pulmonary vein shown. Drawn by G. H. A. Rech.

the cephalic end and another at the caudal extremity. These mark the points of emergence of the cervical and of the abdominal air-sacs. Figure 15 A and B, from a specimen of the last half of the seventh day, show the surface of the left lung and of the right lung of the same embryo. Early on the seventh day the abdominal air-sac projects beyond the border of the lung proper, but the cervical lags behind the abdominal in its development. These are the first two sacs to develop and the others follow shortly except that the posterior intermediate sac is the last to emerge outside the lung wall. The course of the pulmonary artery and of the pulmonary vein is shown in both lungs.

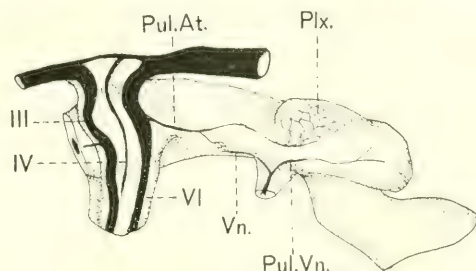


Fig. 14 Lung territory of a well injected specimen of the early seventh day of development. Note especially the laryngo-tracheal artery and its capillary network succeeded by a vessel that connects with the trunk of the united pulmonary veins.

The net work of blood vessels of the anterior dorsal surface of the lung was not easily seen in this specimen, partly on account of imperfect injection and partly because the outer covering is thickened, but in heavily injected specimens, the network is seen (as in fig. 14) to occupy the dorsal anterior half of the lung.

The eighth and ninth days are important periods in the embryonic development of the lung, not only on account of internal changes, but also because the air-sacs emerge, and on the ninth day of development, project beyond the surface of the lung, and thus one of the characteristic structural features of the bird lung is established.

Figure 16, A and B, shows the surface appearance of the lungs on the ninth day of development. The lung has increased in

dimensions dorso-ventrally and when viewed from the side is rectangular in outline. It also occupies a more lateral position in the thoracic cavity. The lung has begun to press against the ribs and exhibits shallow furrows where the lung substance has grown around the bodies of the ribs. The five air-sacs, two on the anterior and three on the ventral margin, are formed and project beyond the surface of the lung. As indicated above, the

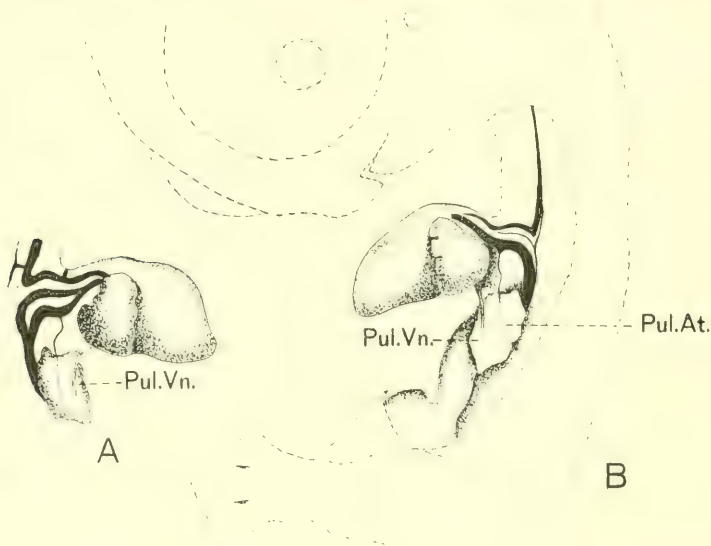


Fig. 15 (A) Dissection of the left side of an embryo during the last half of the seventh day of incubation, the fourth aortic is much atrophied on this side. (B) Heart and right lung of the same specimen. The fourth aortic arch is larger on this side and separated from the third. Projections of the cervical and abdominal air-sacs are exhibited. Drawn by G. H. A. Rech.

posterior intermediate is the last of the air-sacs to expand and project beyond the lung wall. The cervical, and interclavicular sacs are smaller, but both project from the lung wall earlier than the posterior intermediate. The part of the interclavicular sac showing in figure 16 is only the lateral moiety of the sac, the mesial moiety, which at this stage is separate and independent, can not be seen from this aspect.

Well injected specimens of this age show that the blood supply predominates in the dorsal region of the lung. After the air-sacs are well projected their walls are relatively thin and they do not exhibit any blood vessels that will take the India ink in-

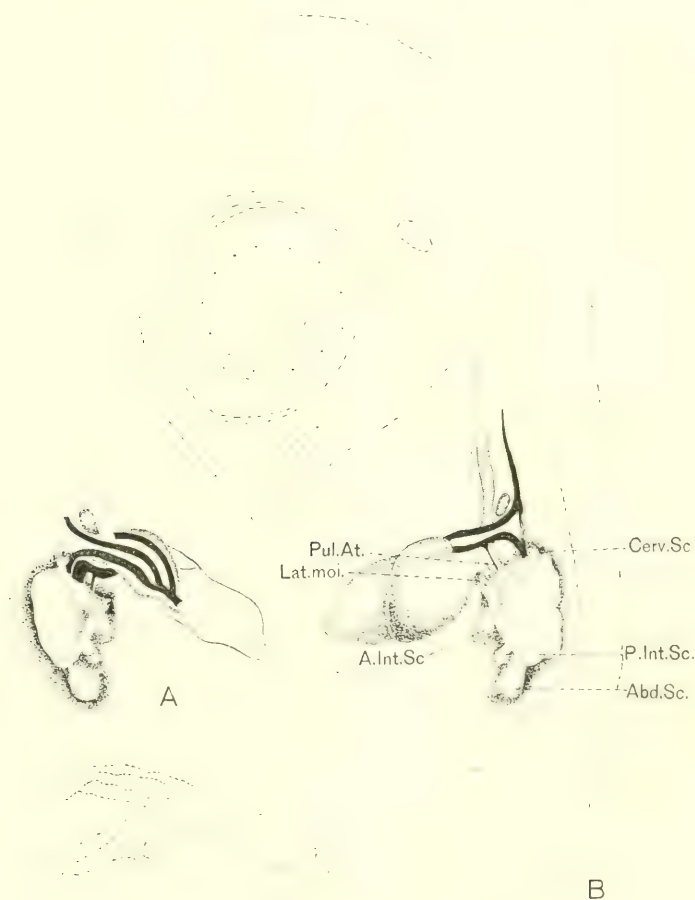


Fig. 16 (A) Dissection exposing the left lung of an embryo of the ninth day of incubation showing five air-sacs projecting from the lung. The mesial moiety of the interclavicular is hidden from view. (B) Heart and right lung of the same specimen. Modified from a sketch by G. H. A. Rech.

jection. As is well known, their blood supply in later stages is derived from arterial branches coming from the aorta. The superficial distribution of blood vessels is shown in figure 17 which is from a specimen somewhat younger than that sketched in figure 16. It is to be understood that the internal plexi of capillaries are extensive and the blood vessels represented in figure 17 are those visible through the translucent walls of the lung and are mentioned here merely as a feature of external anatomy.

Figure 18 is a surface view of both lungs of an embryo at the close of the ninth day of development. The bronchus of the right side has been severed and the right lung rotated so as to expose more fully the mesial facet. The five air-sacs are now well projected beyond the lung wall and in this figure a new structural feature is brought into evidence. This is the mesial moiety (*Mes.moi.*) of the interclavicular air-sac. At this stage it is connected through the interclavicular canal with the anterior intermediate air-sac, and the mesial moiety is widely separated from the lateral moiety. At a later period (fifteenth day, fig. 51) the mesial moiety comes into contact with the lateral moiety and subsequently the two moieties fuse into one sac. In the published sketches of surface views of embryonic stages (with the exception of a figure by Selenka, '66) the mesial moiety has not been represented. It is commonly hidden from view between the two lungs. There are however some published sketches of section of the lungs, as Lillie, '08, Juillet, '12, etc., in which it has been represented as a forward projecting diverticulum of the anterior intermediate air-sac, but in these sections it has heretofore been interpreted as a portion of the anterior intermediate sac. Also in a diagram of Juillet, '12, (cf. his fig. X, p. 313) the mesial moiety of the lung of the embryonic chick is represented to the exclusion of the lateral moiety. In the genus *Larus*, even in the adult, a separate lateral sac of the interclavicular is present in addition to the mesial portion of that sac (Juillet's fig. XVII, p. 351). For the further history of these moieties of the interclavicular air-sac see figures 47, 49, 50 and 52 and the accompanying comments.

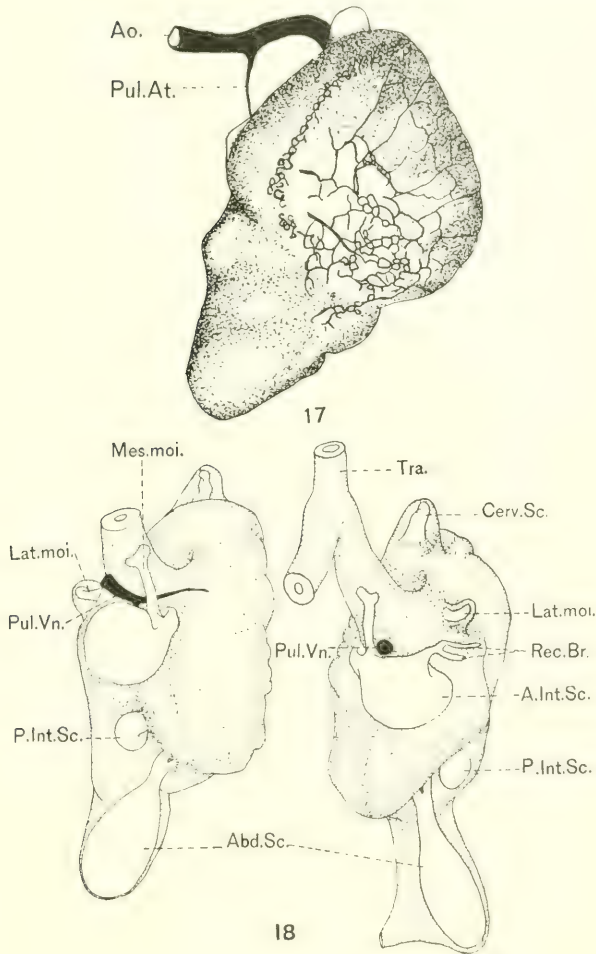


Fig. 17 Camera tracing of the capillary network on the dorsolateral surface of the left lung of an embryo at the close of the eighth day of incubation. Drawn by Mary Head.

Fig. 18 Surface view of the lungs of an embryo at the close of the ninth day of development. Right bronchus cut and lung rotated so as to expose the mesial surface. *Abd.sc.*, abdominal air-sac; *A.intr.sc.*, anterior intermediate air-sac, with primordia of recurrent bronchi (*Rec.Br.*); *Cerv.sc.*, Cervical air-sac; *Lat.moi.*, lateral moiety and *Mes.moi.*, mesial moiety of the interclavicular air-sac; *P.intr.sc.*, posterior intermediate air-sac.

The obvious external feature of the tenth day is the indentation of the ribs on the dorso-lateral border of the lungs. Figure 19 shows a dissection exposing the right lung and adjacent organs of an embryo at the beginning of the eleventh day. There are four well marked indentations of the ribs. As mentioned above, during the ninth and tenth days the lungs undergo a change in position passing from a more ventral to a more dorsal position, and in so doing come close against the ribs, and the dorsal margin of the lung comes to lie along the vertebral column. The air-sacs are enlarged and the proximal ends of the two posterior ones are constricted to form a sort of neck. The abdominal air-sac has increased relatively faster than the others.

The trunks of recurrent bronchi are also shown in connection with the two posterior air-sacs. The recurrent bronchi are the most important structural feature that we have yet had occasion to mention. They begin on the ninth day as buds from the proximal ends of the abdominal and the posterior intermediate air-sacs, and, later, the other air-sacs, with the exception of the cervical, give rise to similar outgrowths. They are destined to develop ramifications that anastomose with parabronchi in various parts of the lung and play a very important part in its physiology. They are so important that they receive separate treatment in section 3 to which reference should be made for figures and further details.

Figure 20 is a diagram made from a study of the left lung of an embryo, incubated $9\frac{1}{2}$ days, to show especially the relations of the mesial and the lateral moieties of the subbronchial sac at this stage of development. In the preceding sketches (except figure 18) the mesial moiety of the interclavicular sac has not been shown chiefly because the sketches were executed before we had learned to look for the two moieties of this sac, and, further, the aspect from which the specimens were drawn did not bring the mesial moiety into view.

The diagram (fig. 20) was made from observations by reflected as well as by transmitted light and the connections of the air-sacs with the bronchi are indicated. On the lateral border of the lung is seen the lateral moiety (*Lat.moi.*) of the interclavic-

ular sac connected with the transverse branch of the first entobronchus. The mesial moiety (*Mes.moi.*) arises in connection with the anterior intermediate air-sac, and these two have a common opening into the third entobronchus. The distal end of the mesial moiety is forked and partly encircles the main bronchus.

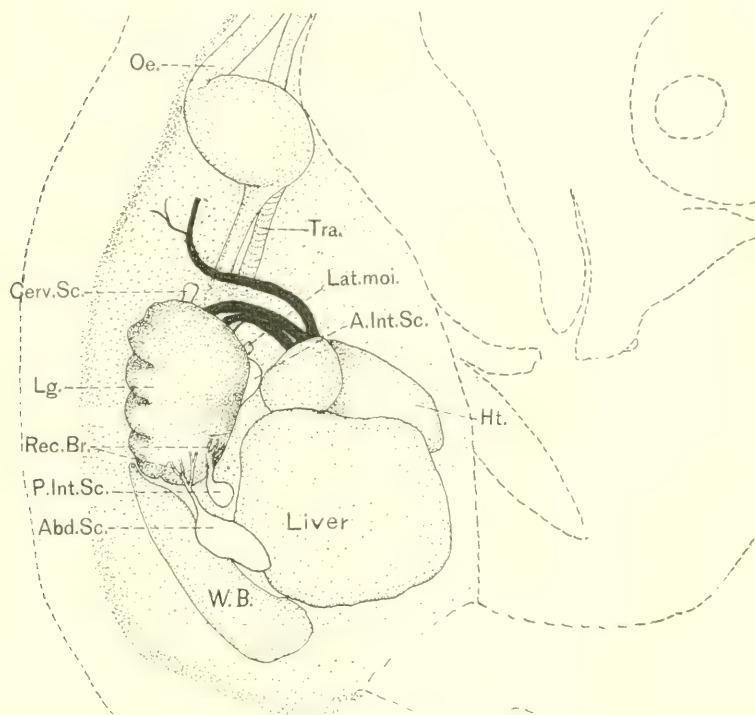


Fig. 19 Dissection exposing the right lung and adjacent viscera of an embryo at the close of the tenth day of incubation. Shows indentations of the ribs and recurrent bronchi from the abdominal and posterior intermediate air-sacs.

In embryos $10\frac{1}{2}$ days old the air-sacs exhibit the very interesting condition (figs. 40 and 47) of recurrent bronchi springing from all the air-sacs except the cervical (which never has any), and, further, that the interclavicular sac is derived from two moieties on each lung. These are the lateral and mesial moieties already referred to. Details in reference to the union of these

four moieties to form the single interclavicular sac of the adult are brought out in section 3 of this paper.

The significant external features of the lung are now established and subsequent changes show, chiefly, increase in size of the lungs, a relatively larger increase of dimensions of the air-sacs and the development of recurrent bronchi, which on the fifteenth day anastomose with parabronchi.

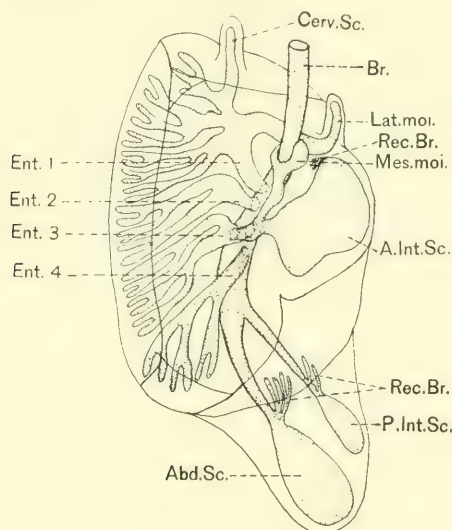


Fig. 20 Diagram based on the dissection of the lung of an embryo incubated $9\frac{1}{2}$ days. Designed to show the lateral and mesial moieties of the interclavicular sac and the connections of air-sacs with the bronchial tree.

Figure 21, a latero-mesial view, shows the relative dimensions of the lung and its air-sacs on the twelfth day of development. There are four well marked indentations of the ribs. The trunks of recurrent bronchi are indicated as developing from the abdominal and posterior intermediate air-sacs, and also from a small accessory sac between them. This accessory sac is apparently cut off from the posterior intermediate and our observations incline us to the belief that in this specimen it is connected with the fourth laterobronchus. Schulze ('11) has called attention to it (and its recurrent bronchi) as commonly present in *Ciconia*.

Dissoura and other birds and also as occurring infrequently in the chick. At the base of the recurrent bronchi of the posterior intermediate and of the accessory sac is an enlargement or pocket



Fig. 21 Latero-mesial view of the left lung and air-sacs of an embryo of twelve days incubation. The air-sacs exhibited include the lateral and mesial moieties of the interclavicular and an accessory sac between the posterior intermediate and the abdominal sacs. Recurrent bronchi from the posterior three sacs are shown. Similar bronchi are present on the anterior intermediate and lateral moiety of the interclavicular but hidden from view.

(Basaltasche), which Schulze has pointed out as a characteristic structure at the base of the recurrent bronchi of the different air-sacs.

The connection between the anterior intermediate and the mesial moiety of the interclavicular sac is well exhibited. The fork (shown in other figures) on the distal end of the mesial moiety is not visible owing to the position in which the specimen is viewed. It curves around the main bronchus away from the observer. The common opening (interclavicular canal) into the third entobronchus, of the anterior intermediate and the mesial moiety is well exhibited in this specimen.

The external appearance of the lung on the thirteenth day of development is shown in figures 22 and 23, in which the lung of the left side is sketched as exposed by dissection within the thoracic cavity (fig. 22) and both right and left lungs sketched on a larger scale as removed from the thorax (fig. 23). The points to be noted are: the lateral position of the lungs in the thoracic cavity; the deep indentations of the ribs on the latero-dorsal border; the presence of five air-sacs with the stems of the recurrent bronchi from the abdominal and the posterior intermediate air-sacs. The mesial moiety of the interclavicular sac is hidden by the curvature of the lungs. On the surface of the right lung is seen a clear space between the abdominal and the posterior air-sacs. This probably corresponds to the accessory sac of figure 21.

The most significant points regarding the later stages is the assumption of the adult condition of the air-sacs and the further development of the recurrent bronchi. These matters are dealt with in section 3.

The examination of the series of figures described above shows that the external anatomical landmarks of the embryonic lung consist chiefly of its size, shape, the emergence of the embryonic air-sacs, the development of the trunks of recurrent bronchi and the superficial distribution of blood vessels.

The more important internal features embrace the development of the bronchial tree, including the intrapulmonary parts of the recurrent bronchi, and the development of air capillaries around the parabronchi. These matters will now receive consideration.

2. THE DEVELOPMENT OF THE BRONCHIAL TREE

In this section will be described the intrapulmonary phenomena of development of the bronchial tree. Although, morphologically speaking, the air-sacs and recurrent bronchi are parts of the bronchial tree they will be considered separately.



Fig. 22 Dissection exposing the left lung territory of a chick embryo of thirteen days incubation. Modified from a sketch by G. H. A. Reeh.

As Campana pointed out, there is no 'bronchial tree' of the adult bird's lung in the sense in which this designation is used for mammals. No bronchial twigs of the adult avian lung terminate blindly. On the contrary, there is established a network of

intercommunicating passages forming bronchial circuits. With this qualification we may retain the convenient term 'bronchial tree' as applying to the central trunk and its larger branches with their subdivisions, but remembering that the terminal twigs do not end as in the bronchial tree of mammals. The resem-

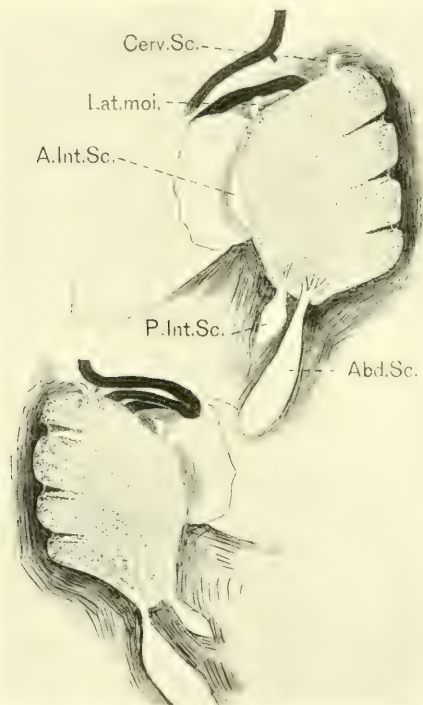


Fig. 23 Enlarged view of the lungs of the specimen sketched in figure 22 removed from the body. Five air-sacs are shown. The mesial moiety of the interclavicular not visible. Modified from a sketch by G. H. A. Rech.

blance is closer to the capillary connection between arteries and veins than to a tree.

From their earliest formation, the lung pouches are lined by endoderm and this internal cavity is the basis from which hollow buds arise to form branches of the bronchial tree. The endodermal tube lies in a layer of mesenchyme that is bordered on the surface towards the pleuro-peritoneal cavity by a well defined

layer of mesothelium. Accordingly, the external boundaries of the lung are formed by a wall of mesoderm which gives no indication of the internal configuration of the endodermal lining. The formation of the branches of the tree must be traced by reconstructions or by air injections, since the study of the lungs as transparencies, without such injections, is very limited in its application and does not show the outgrowths with any degree of completeness as to detail.

At the 96-hour stage the simple cylindrical tube of endoderm extends into the mesenchyme of the lung primordium and is slightly expanded at its distal extremity (fig. 24, A and B). The trachea has already begun to differentiate from the pharyngo-tracheal groove, and in a few hours' time is definitely formed. The stomach enlargement (ventriculus) pushes on the left lung so as to throw it out of alignment and give an appearance of asymmetry to the lungs.

At the proximal end, a short portion of the lung tube lies outside the boundary of the mesenchymal swelling of the lung pouch and forms the primordium of the extra-pulmonary bronchus (fig. 24).

By the 100-hour stage the trachea is distinctly formed (fig. 25); it is of larger calibre than the bronchi that connect with it. The oesophagus curves abruptly from it in the dorsal plane, and then, with a more gentle curvature, it passes caudad for some distance and finally curves ventrally and passes into the stomach enlargement between the lungs.

On the second half of the fifth day of development a spindle-shaped expansion arises within the lumen of the lung tube (fig. 26). This is a convenient anatomical landmark of the central lung tube of the embryo and may be designated as the embryonic vestibulum. It does not correspond however to the vestibulum of the adult, into which the entobronchi open, neither is it the genetic forerunner of the adult vestibulum. It is a relatively thin-walled dilation of the central lung tube located further caudad than the adult structure of that name. Figure 26 shows its dimensions at four days and twenty hours' incubation. Its position varies from about two-fifths to two-thirds the space from

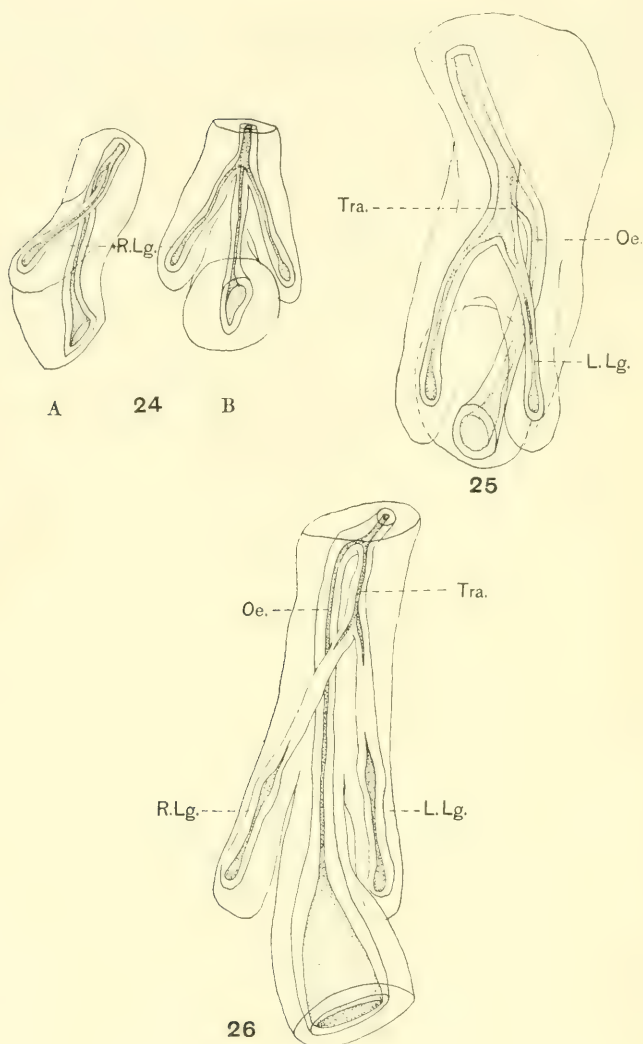


Fig. 24 Side and ventral view of the central lung tube of an air injected specimen of the 96-hour stage. Shows an early stage in the development of the trachea.

Fig. 25 Ventro-lateral view of the air injected lungs of an embryo incubated 100 hours. The trachea is definitely formed.

Fig. 26 Cedar oil transparency of the lungs of a specimen incubated 4 days 20 hours, showing the expanded embryonic "vestibulum" and the occluded portion of the bronchus.

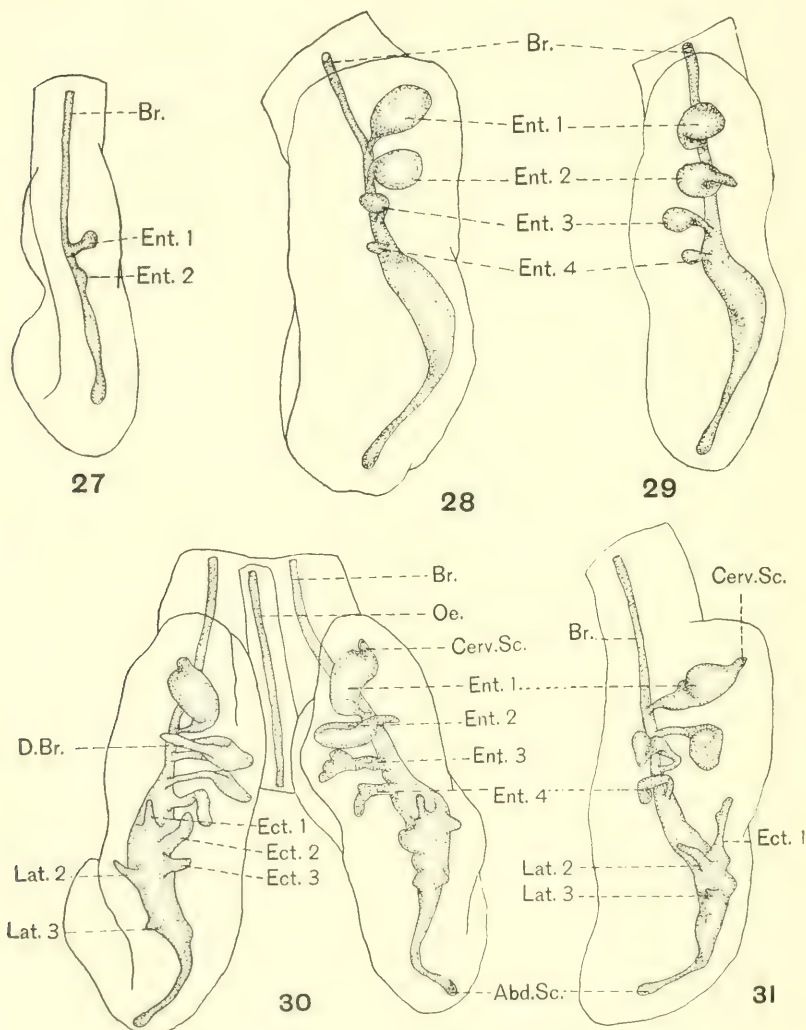
the anterior to the posterior end of the lung tube. The ectobronchi arise from this expanded region. Later the more cephalad region of the lung tube increases in diameter and, owing to unequal growth, the embryonic dilation merges into the rest of the lung tube and is no longer conspicuous. The region of the adult vestibulum is, in reality, occluded at this stage of development. As shown in figure 26, the greater part of the future lumen between the trachea and the anterior end of the lung becomes occluded early on the fifth day and this condition persists throughout that day of incubation. This occluded portion embraces the region of the adult vestibulum.

The embryonic dilation referred to was first figured by Selenka in 1866. Its rise served to divide the central lung tube into three regions—an interior, a middle and a posterior. The lung tube as a whole is in the shape of an elongated S and the dilatation of the middle region arches so as to come close to the dorsal surface of the lung.

Shortly after the appearance of the embryonic vestibulum, the primary lung tube, early on the sixth day, begins to give off buds which form the beginnings of the secondary branches of the bronchial tree. The first bud to be formed (fig. 27, *ent.* 1, 5 days, 9 hours) is from the internal (mesial in the adult) wall of the intrapulmonary bronchus. This is the primordium of the first entobronchus. Behind this (in the same figure) is the smaller bud of the second entobronchus. It will be noted that both are in front of the embryonic vestibulum.

Two similar hernia-like enlargements follow in quick succession and form the primordia of the third and fourth entobronchi. It results that, at the stage of five days twenty hours' incubation there are present four bladder-like outgrowths (fig. 28, *ent.* 1, 2, 3, 4) which are connected with the bronchus by slender stalks. The third and fourth have their attachment to the bronchus somewhat more mesially than do the first and second. These four entobronchi, although arising on the internal wall, curve around the bronchus as they grow so as to occupy the ventral face of the lung.

Since the bronchial branches are known under a variety of names, it will be advantageous before proceeding further to indicate the nomenclature employed in this paper. The different secondary outgrowths from the primary lung tube have the same histological structure, but they vary in position, in size, and in the profusion and distribution of their branches.



Sappey, in 1847, described two principal kinds, the diaphragmatics (bronches diaphragmatiques), branching towards the ventral face, and the costals (bronches costales), extending dorsally below the ribs.

In 1875, in his extensive memoir, Campana designated three categories: primary, secondary and tertiary bronchi. The primary (*La bronche primaire, ou Souche*) is the central lung tube commonly called mesobronchus. He distinguished four groups of secondary bronchi which we have adopted with some modifications. Campana's divisions are: (1) The system of five large divergent bronchi (the diaphragmatiques of Sappey); (2) The system of eight internal bronchi (the costales of Sappey); (3) The system of six external bronchi, for which we have adopted Schulze's term of laterobronchi (*lateribronchi*); (4) The system of posterior or dorsal bronchi for which we employ the term dorsobronchi (*dorsilateribronchi* of Schulze).

The tertiary bronchi, or terminal branches of subdivisions of the secondary, are commonly known as parabronchi or air-pipes.

Huxley, in 1882, introduced the terms mesobronchium for the central lung tube, and ento- and ectobronchia for the diaphragmatics and the costals; we have adopted these simplified terms.

While on anatomical grounds some critical objections may be made to certain groups, we have, nevertheless, purely for descriptive purposes adopted the following terminology:

For the primary lung tube, mesobronchus with its three divisions, anterior, middle and posterior.

Fig. 27 Air injected lung of an embryo of 5 days 9 hours incubation showing the bud of the first entobronchus and the beginnings of the second.

Fig. 28 Lateral aspect of the air injected lung of an embryo of 5 days 20 hours incubation.

Fig. 29 The same specimen rotated so as to be viewed from the dorso-lateral aspect. This figure and the preceding show the establishment of the four entobronchi in front of the dilatation of the mesobronchus.

Fig. 30 Dorsal aspect of both lungs of a chick embryo of 6 days 6 hours incubation. Shows the appearance of the bronchial tree when injected with air as explained in the text.

Fig. 31 Lateral view of the air injected lung of an embryo of 6 days 6 hours incubation. This specimen was slightly younger than the one sketched in figure 30. Shows the four entobronchi and the buds for the first four ectobronchi.

The secondary bronchi, or the original branches, from the central lung tube are designated under four divisions: entobronchi (ventribronchi of Schulze); ectobronchi (dorsibronchi of Schulze); laterobronchi and dorsobronchi.

For tertiary bronchi, the terminal branches of the subdivisions of the four kinds of secondary bronchi, we use the term parabronchi. These are tubes of uniform calibre and unite the different systems of bronchi into bronchial circuits.

In addition to the above, coming from the air-sacs, are the recurrent bronchi of Schulze and Juliet and the air capillaries that are radially arranged around the parabronchi.

After this digression on the terminology we continue the description of embryonic stages. When the four entobronchi are well started, another series of hernia-like buds arise early on the seventh day. These are the primordia of the ectobronchi and they arise from the wall of the embryonic vestibulum. While in the chick there are usually six, in birds in general they vary from six to ten. It should also be noted in passing, that the four entobronchi enumerated in the chick, may in other birds be as many as six (pelican, Schulze).

The first ectobronchus springs from the widest part of the embryonic vestibulum. By six days six hours it is already of considerable length (figs. 30 and 31) and projects forward and slightly laterally. The second, third and fourth ectobronchi are at this stage only papillate buds that protrude dorso-mesially (dorsally in the adult) from the vestibular wall and caudad to the first.

Figure 31, from a different specimen of the same age as figure 30, shows the first ectobronchus in a more favorable position.

A fifth and sixth ectobronchus are subsequently developed from the wall of the mesobronchus, but further consideration of both ento and ectobronchi will be deferred to a later page.

Reference to figure 32, which is a sketch of the condition of the bronchial tree in the last half of the seventh day (six days, twenty hours), discloses another series of incipient secondary branches from the central lung tube. These are formed on the lateral wall of the embryonic vestibulum and were called by

Campana ('75) 'bronches secondaires externes.' Although they do not attain in the adult lung the dimensions of ento- and ectobronchi they are, nevertheless, coordinate with them (1) in that they arise directly from the central lung tube; (2) that they are the principal branches supplying the lateral part of the lung; (3) that they make their appearance about the same time as ectobronchi. Accordingly the term laterobronchi (lateribronchi, Schulze) seems appropriate.

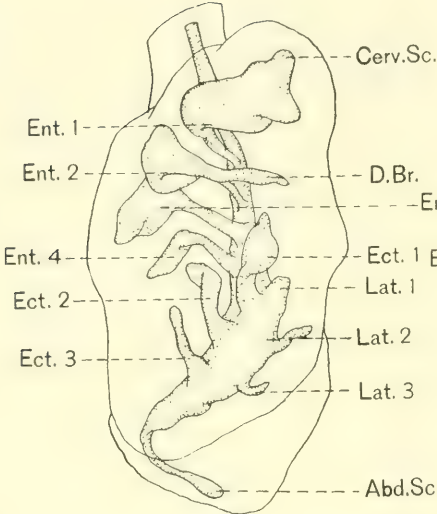
The first three laterobronchi are illustrated in figure 32, *lat.* 1, 2, 3. Subsequently three smaller ones arise caudad to the anterior three making a total of six laterobronchi.

Other relatively small bronchi, somewhat more dorsal in position, arise similarly, but at a later stage. These correspond to Campana's fourth kind of secondary bronchi and we have called them dorsobronchi (dorsilateribronchi of Schulze). Descriptions of the numerous dorsobronchi and of the six laterobronchi will be taken up later.

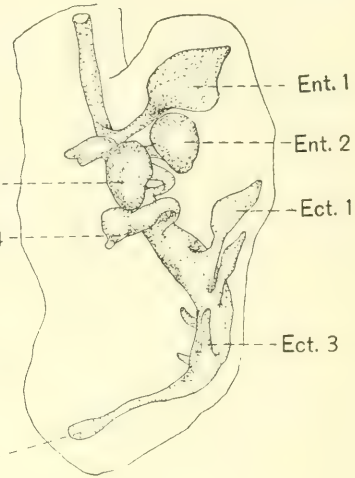
Attention may be briefly directed to the configuration of the central lung tube. It forms a figure resembling an elongated S, the posterior bend of which is more marked. The terminal end is inflated and projects into a protuberance of mesenchymal tissue, and form the primordium of the abdominal air-sac. The central expanded part is the embryonic vestibulum. As indicated above, the embryonic vestibulum is not to be confused with that of the adult.

The bronchial tree from this stage on, continues to grow and its branches to ramify so profusely, that for the sake of clearness it is necessary to describe its subsequent development under separate headings.

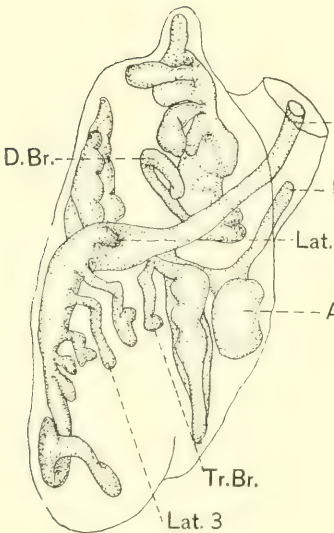
The entobronchi. As noted above, the first entobronchus makes its appearance early on the sixth day of development as a papillate bud from the dorsal wall of the anterior division of the mesobronchus (mesial in the adult lung). In order to maintain a correct orientation the following should be noted. Beginning late on the ninth day the lungs begin to rotate about their longitudinal axis and pass through an angle of about 30 degrees before reaching their adult position. This rotation carries the



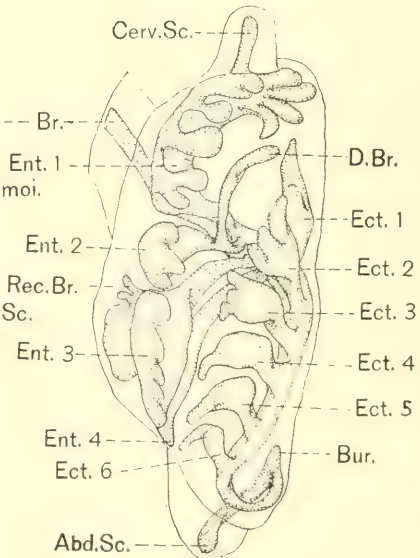
32



33



34



35

previously ventro-lateral border laterad, and the previously dorsal border towards the median plane.

During the last half of the sixth day (fig. 28, 5 days, 20 hours), the slender stalk of the first entobronchus extends dorsally for a short distance, then curves mesially and expands, the trend is again dorsad, and finally, laterad. The enlarged bladder-like extremity lies directly above the bronchus (fig. 29) and somewhat anterior to the point where the stalk joins the bronchus.

Soon the distal enlargement of the entobronchus begins to divide as shown in figures 30 and 32. One bud-like outgrowth (*Cerv.sc.*) extends cephalad and constitutes the primordium of the cervical air-sac. The other bud, visible from this position, extends laterad and ventrad.

It is not, however, until a later period that the divisions of the first entobronchus can be clearly seen. On the ninth day of development (figs. 36 and 37), the first entobronchus exhibits three principal divisions, or branches, each of which is subdivided by lobular branches. The cranial branch (*Cr.br.*) extends forward and bears at its tip the cervical sac (*Cerv.sc.*) which at this period extends beyond the lung wall. The cervical sac is not terminal in the adult as in the embryo, on the contrary, its orifice opens upon the surface of the main part of the cranial branch. The transverse, or lateral branch (figs. 36 and 37, *Lat.br.*) extends

Fig. 32 Dorsal view of the lung of an embryo incubated 6 days 20 hours. Illustrates ento- and ectobronchi and the early condition of laterobronchi.

Fig. 33 Mesial view of the same specimen.

Fig. 34 Ventral view. *Abd.sc.*, abdominal air-sac; *A.intr.sc.*, anterior intermediate air-sac; *Bd.*, bud of recurrent bronchi; *Cerv.sc.*, cervical air-sac; *Dor.*, dorsobronchi; *Dr.*, dorsal ramus of second entobronchus; *Ect.* 1, 2, 3, etc., the corresponding ectobronchi; *Ent.*, 1, 2, 3, 4, the corresponding entobronchi; *Lat.*, 1, 2, etc., the corresponding laterobronchi; *Lat.moi.*, lateral moiety of the interclavicular air-sac; *Mes.moi.*, mesial moiety of the interclavicular air-sac; *P.intr.sc.* posterior air-sac.

Note: Figures 34, 35, 36 and 37 represent the air injected right lung of an embryo of the early ninth day of incubation viewed from different aspects. At this period the principal divisions of the bronchial tree are established, although still in a relatively simple stage of development, and the air-sacs with the exception of the posterior intermediate are well outlined. The reference letters are the same for all figures.

Fig. 35 Dorsal view. (Reference letters as above.)

towards the lateral border and a slender outgrowth at its tip (*Lat.moi.*) foreshadows the lateral moiety of the interclavicular air-sac. The medial branch extends transversely in the opposite direction (fig. 37 *Mes.br.*).

The further development of the first entobronchus consists in the subdivision of its lobes into smaller lobules. These, in turn, elongate and give off branches that develop into the parabronchi of the cephalic region of the lung. The first entobronchus is a

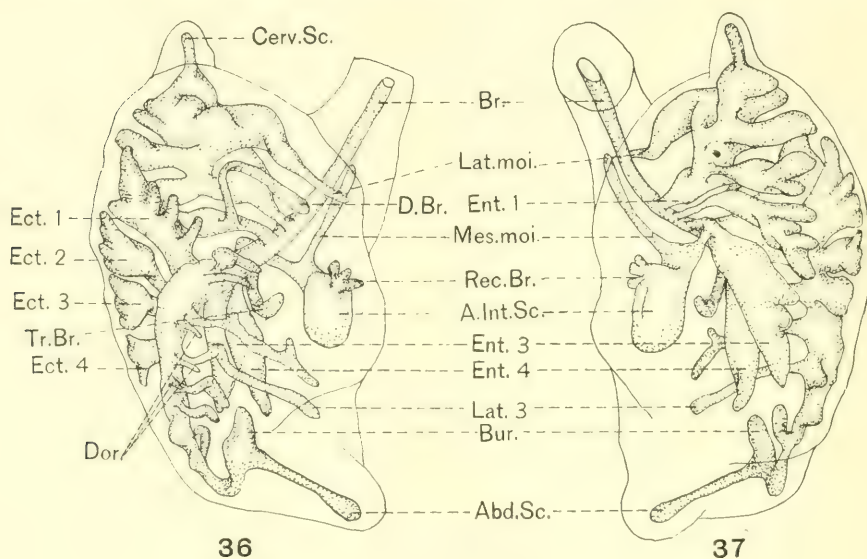


Fig. 36 Lateral view. (Reference letters as in figure 34)

Fig. 37 Mesial view. (Reference letters as in figure 34)

very large and important part of the bronchial passages. Its subdivisions supply the summit of the lungs (fig. 44) and also give rise to two air-sacs, the cervical and the lateral moiety of the interclavicular air-sac.

Entobronchus number two (figs. 28 and 30, *Ent. 2*) has a similar origin to that of number one. It starts about the middle of the sixth day of incubation as a bud from the dorsal wall (mesial in the adult) of the intra-pulmonary bronchus. It grows rapidly, so that, at five days, twenty hours (figs. 28 and 29) it has an

expanded distal end attached by a slender stalk to its point of origin. The stalk first grows mesiad, then enlarges and bends dorsad, and finally, expands into the distal sac mentioned above. This expansion, like that of the first entobronchus, lies directly above the bronchus (fig. 29), but slightly towards the mesial border of the lung. It gives off from its lateral border a slender elongated branch which at six days, twenty hours, crosses the bronchus dorsally (fig. 32) and at its tip bends slightly ventrolateralward. This is the dorsal ramus of the adult second entobronchus. It is first noticeable in the latter part of the sixth day.

Aside from increase in dimensions, but little change occurs in the second entobronchus or its dorsal ramus until the ninth day of development. At this time, as illustrated in figures 35 and 37, the extremity of the entobronchus has divided into two unequal lobe-like branches, the more posterior of which is bifurcated (fig. 37). The dorsal ramus has elongated and taken on a more ventral curve (fig. 37) and is a distinct anatomical feature.

The second entobronchus is smaller and of less wide-spread distribution than the first. The three lobes, formed on the eighth day, subdivided into branches, which on the ninth day, begin to differentiate into parabronchi that ultimately supply the anterior mesial region of the adult lung. The parabronchi of the dorsal ramus supply the interior of the cranial part of the lung between the stem of the first entobronchus and its transverse branch.

Exceptionally the second entobronchus gives off near its base a mesial branch (fig. 38) from which develops the mesial moiety of the interclavicular sac. The mesial moiety, however, as described below, usually has its origin from the third entobronchus. This exceptional point of origin may account for the fact that some observers have ascribed the origin of the interclavicular sac to the second entobronchus while others have claimed that it arises from the third entobronchus.

The third entobronchus arises (fig. 28) somewhat more mesially on the intra-pulmonary bronchus than the first two. On the second half of the sixth day it extends dorsally a short distance,

then enlarges and bends caudally, descending on the mesial side of the central lung tube so that the expanded distal end lies at the side of the main bronchus, not above it, as is the case with entobronchi numbers 1, 2.

Figures 30, 32 and 33 represent the beginning of a bud projecting ventrally from the third entobronchus which is to play an important part in the later history of the lung. This hollow bud

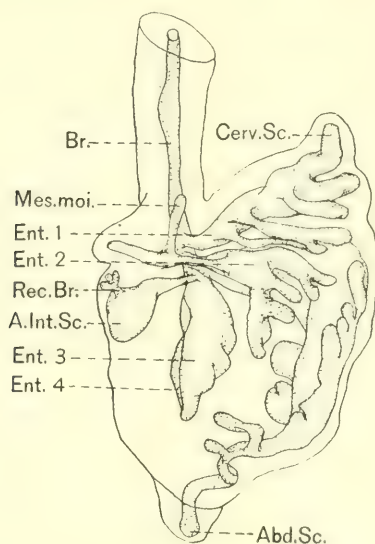


Fig. 38 Dorso-mesial view of right lung of an embryo slightly younger than the one sketched in figures 34 to 37. Illustrates the exceptional position of origin of the mesial moiety of the interclavicular air-sac, from the second instead of the third entobronchus.

gives rise to two branches, one cephalad and the other ventrad. The more cephalad branch forms the mesial moiety of the interclavicular air-sac (figs. 33 34); the ventrad, bladder-like portion, differentiates into the anterior intermediate air-sac. By the close of the eighth day of incubation both these air-sacs project beyond the boundary of the lung.

It should be noted that under the exception indicated above, namely, when the second entobronchus gives rise to the mesial

moiety of the interclavicular sac, the branch of the third entobronchus just described does not divide and produces only the anterior intermediate air-sac.

The other division of the third entobronchus elongates and expands caudo-dorsally until, early on the ninth day, its dorsal face becomes tri-lobed (fig. 37). At the ten-day stage these lobes branch and later give rise to the parabronchi of the middle portion of the mesial lung facet.

In the adult, the third entobronchus extends obliquely toward the caudal extremity of the lung on its ventral face (fig. 44). From its medial border six or seven parabronchi are given off and on its lateral border is a transverse branch connecting it with the fourth entobronchus.

The fourth entobronchus has an embryonic history very similar to that of the third, but it is not connected with any of the air-sacs. Its origin is on that part of the mesobronchus where the swelling of the embryonic vestibulum begins, or where the anterior part of the mesobronchus merges with the middle part. Its orifice is somewhat more mesial in position than that of the third entobronchus.

Shortly after the beginning of the seventh day the fourth entobronchus is short and extends mesially with an expanded end which is directed caudally (fig. 30).

On the eighth day a branch is budded off very close to its base, which early on the ninth day forms a sickle (figs. 34 and 37) with its point directed laterally. In the adult this sickle-shaped branch becomes the great transverse branch, extending obliquely across the ventral face of the lung and forming a prominent anatomical landmark (fig. 44). Campana ('75) enumerated this large transverse branch as a fifth entobronchus, but since it has no direct connection with the central lung tube, even in the embryo, we have, as most previous observers, considered it a branch of the fourth entobronchus.

The principal trunk of the fourth entobronchus extends caudally and mesially in a course parallel to the caudal branch of the third entobronchus. The territory included between the main trunk and the transverse branch is overrun with small

branches springing from both the main division and the transverse branch.

It is evident that the entobronchi begin very early to play a leading part in the formation of the bronchial tree and although limited in number they make a large showing on the metal casts of the adult lung. Generally speaking, the entobronchi of the adult are distributed on the ventral surface of the lung as shown in figure 44. The parabronchi that spring from their branches bend around the mesial border of the lung and curve on to the dorsal surface where they meet parabronchi coming from the ectobronchi and thus a connection is established between these opposed bronchi and the characteristic bronchial circuits.

The ectobronchi. The ectobronchi are outgrowths from the central lung tube arising somewhat later than the entobronchi and more mesial in position upon the wall of the lung tube. These are the bronches costales of Sappey and the secondaires internes of Campana. While the ento- come from the anterior division of the mesobronchus, the ectobronchi spring from the wall of the embryonic vestibulum in the middle division of the central lung tube.

The first (fig. 30, *Ect.* 1) arises as a forward and upward projecting bud from the anterior part of the expanded region of the tube at the beginning of the seventh day. By rapid growth it becomes elongated, and, at six days, twenty hours, may be seen the first indication of branching in the form of a ventral bud springing from the distal end of the ectobronchus (fig. 32). The two lobes produced in this manner rapidly increase in size and give rise to other lobe-like branches. As illustrated in figure 36 there are five such lobules at the beginning of the ninth day. The two most dorsal are the result of the division of the original dorsal lobe, and the three ventrally placed lobules come from the other division of the first bifurcation.

These lobular outgrowths foreshadow the parabronchi of the anterior lateral and of the dorsal region of the lung. By the close of the ninth day the lobules have greatly increased in number and, on the tenth day, show the beginnings of some parabron-

chi (fig. 39, *Par.*). The original bifurcation can be readily seen in the later stages (fig. 40).

The second ectobronchus (figs. 31 and 33, *Ect.* 2) arises early on the seventh day of development as a bud from the dorso-mesial surface of the vestibulum (embryonic). Its point of attachment is slightly more mesial than that of the first ectobronchus and at first its course is nearly parallel with the first ectobronchus, but in late stages it becomes transverse. A bud arises on its distal extremity towards the close of the seventh day but there is no definite branching until early on the ninth day at which time the distal end is divided into three lobes (figs. 35 and 36). These lobules are the first indications of the subdivisions whose branches become parabronchi as shown in figure 39. In the adult the parabronchi of the second ectobronchus supply the dorso-medial face between the third and fourth ribs.

The embryonic history of the remaining four ectobronchi is so similar that only brief descriptions are required. It is sufficient to note that each has its origin slightly more mesially than the preceding on the dilatation of the lung tube and, after branching has begun, they incline more towards the caudad border of the lung. Beginning with ectobronchus number three this caudal bending becomes more marked. In the adult their parabronchi supply the mesial region of the posterior half of the lung. The parabronchi that originate from the sixth ectobronchus spread out in such a manner as to be distributed to the dorso-caudal part of the lung.

None of the ectobronchi have any direct connection with air sacs.

A seventh ectobronchus is sometimes present in the chick and becomes connected with recurrent bronchi from the abdominal air-sac.

As to their number in birds in general, Schulze has pointed out that they vary from six to ten and that seven is the usual number.

The laterobronchi. The laterobronchi correspond to those designated 'bronches secondaires externes' by Campana. Six

laterobronchi are developed, but not simultaneously, and they arise as buds from the lateral wall of the embryonic vestibulum. The first two (fig. 30, *Lat. 2* and 3) arise at the stage of six days six hours. These are, in reality, numbers two and three—num-

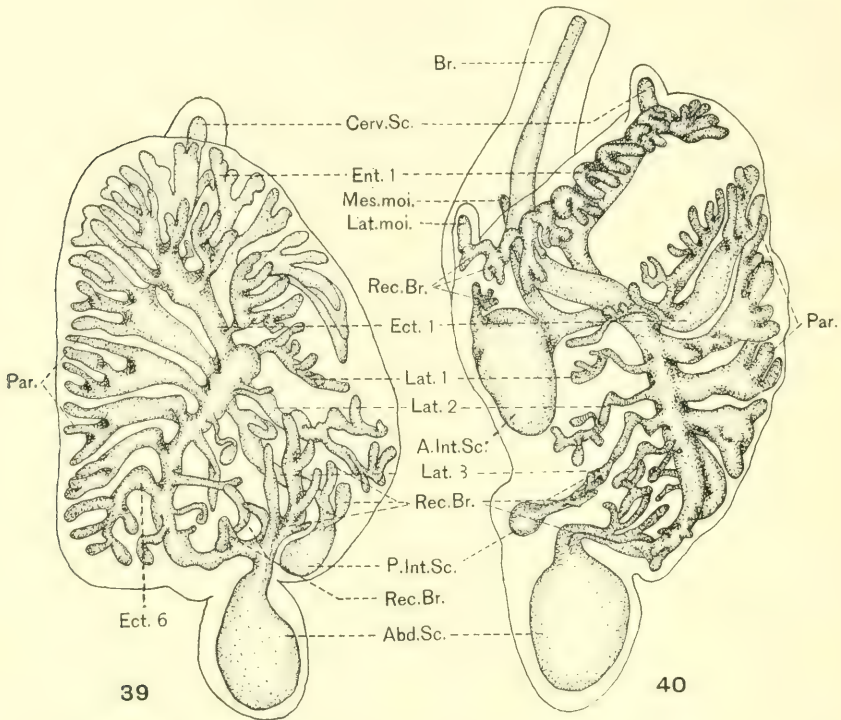


Fig. 39 Latero-posterior view of the right lung of late ninth day of incubation. Shows the beginning of recurrent bronchi on the abdominal and the posterior air-sacs.

Fig. 40 Lateral view of the left lung of the same specimen. Illustrates the five air-sacs and primordia of recurrent bronchi of the four air-sacs possessing them in the adult. *Rec.Br.*, recurrent bronchi, other reference letters as under figure 35.

ber one arising later in front of number two. They elongate in the latero-ventral plane, so that near the close of the seventh day they are distinctly prominent (fig. 32, 6 days, 20 hours). At the beginning of the ninth day (fig. 36) both two and three

make a sharp bend caudally and their distal extremities extend to the lateral border of the lung. Just before reaching this wall, the second latero-bronchous bifurcates, and later forms a number of divisions which result in supplying the middle part of ventro-lateral lung region with parabronchi (figs. 39 and 40).

The third laterobronchus does not divide like the second, but projects beyond the lung wall and forms the primordium of the posterior intermediate air-sac (figs. 39 and 40). This sac makes its appearance at the beginning of the ninth day.

Campana says that this air-sac arises on the second laterobronchus, but he did not study development and a correct understanding is possible only by following embryonic stages.

A number of branches are later given off, from the third laterobronchus between the air-sac and the point of connection of the laterobronchus with the mesobronchus. The first branches of this kind appear on the tenth day and the others later. These branches which extend ventro-laterally, must not be confused with the dorsally and forward projecting buds from the air-sac itself (fig. 40). These latter are the recurrent bronchi and will receive separate consideration on a following page.

We may now consider the first laterobronchus—so-called because it is the most anteriorly situated, although arising later. It is first distinguishable on the second half of the seventh day as a boss-like bud from the antero-lateral wall of the embryonic vestibulum (fig. 32, *Lat.* 1). It grows forward a short distance and then turns ventral (fig. 34). Later a number of branches are given off its anterior and lateral surfaces which produce short parabronchi that eventually anastomose with the deep-lying branches of the transverse branch of the first entobronchus and with parabronchi of the dorsal ramus of the second entobronchus.

The side branches of this laterobronchus anastomose also to some extent with the anterior tips of the recurrent bronchi of the abdominal air-sac.

Laterobronchi numbers four and five take their origin from the posterior division of the mesobronchus, just caudad to the expanded part or embryonic vestibulum. They appear during the eighth day of incubation, and at the beginning of the ninth day

(fig. 36, *Lat.* 4 and 5), they follow a course parallel to that of laterobronchus number three. In their subsequent history they form branches by the customary bifurcation and give rise to parabronchi in the posterior lateral region of the lung.

A small inconspicuous bronchus arises more caudally on the mesobronchus, and although it resembles quite closely some of the larger dorsobronchi, we have considered it as the sixth laterobronchus (fig. 36).

The dorsobronchi. These embrace the bronchi of Campana's fourth division of secondary bronchi and by him designated "bronches posterieurs ou dorsales" (dorsilateribronchi of Schulze, '10). They are smaller and more numerous than the other bronchi arising from the central lung tube. Their orifices are somewhat variable as to position, and some members of the group arise on the stems of the latero- and ectobronchi, and this imparts to them the character of being transitional between the bronchi of the second and of the third order. On strictly anatomical grounds those that spring from the latero and ectobronchi are of the third order, but there are always many, especially of the smaller ones (and frequently large ones), that connect directly with the mesobronchus. Campana thinks that it introduces a useless morphological complication to exclude them from the group of the secondaries. On account of their transitional character and their (sometimes) connections with latero and ectobronchi, Juillet sets them at one side. Our observations, however, lead us to agree with Campana, that, after recognizing that some of them merge into bronchi of the third order, and that they are so small as to resemble parabronchi, nevertheless, there are always a considerable number arising from the walls of the mesobronchus, and therefore, they should be retained in the group of the secondaries.

So far as we are aware no previous embryological observations have been recorded on these bronchi.

There are two groups of the dorsobronchi, four or five larger ones and approximately twenty smaller ones. Figure 36 exhibits three small spur-like projections from the lateral side of the mesobronchus just dorsal to the bases of the first, second, and third

laterobronchi. These are the beginnings of the larger or principal dorsobronchi and they arise on the eighth day of incubation. The stage figured shows their degree of development early on the ninth day.

After this stage additional buds appear, in rather rapid succession, within the space on the wall of the mesobronchus between the bases of the latero- and ectobronchi and, also, more caudad. By the eleventh day so many have developed that, when they are injected with air, they tend to obscure the other air passages of the lungs. Accordingly, all but the larger dorsobronchi have been omitted in the sketches subsequent to the ninth day, in order not to confuse the more important features of the sketches.

In the adult lung they form a very important area on the dorsal surface of the lung which will be described later. As illustrated in figure 55, the bases of the dorsobronchi are arranged roughly in three rows. The middle row is composed of the larger ones, which are the first to develop, and arranged, in a general way, alternating with those of the middle row, mesially and lateral to it, are the more numerous smaller ones. In the diagram (fig. 55) the bases of twenty-one dorsobronchi are shown—five of the larger, opposite the bases of the laterobronchi, and sixteen of the smaller ones. This diagram is made from the study of a Wood's metal cast in which the dorsobronchi were clipped off at their bases. There is individual variation as to the total number present as well as to the pattern of their arrangement on the mesobronchial wall. Campana cites a number of variations in this arrangement. We have examined specimens with twenty-five dorsobronchi—five larger and twenty smaller ones—but to what limit the number of smaller ones may go in the adult we have not determined.

These dorsobronchi occupy a position along the dorsal wall of the central lung tube from the stem of the first ectobronchus to the posterior end of the mesobronchus,

The branches of the dorsobronchi project towards the dorsal surface of the lung, and arriving there, their parabronchi form a well-marked, nearly circular area, or network, in the center of the dorsal face. After the tenth day this area can readily be

seen from surface views of untreated embryo lungs. Figure 41 represents the condition at the age of ten and one-half days. The small circles in the middle of the dorsal area represent end views of the dorsobronchi and their subdivisions. At the posterior and lateral parts of the circular area are several dorso-bronchi curved in such a way as to show other side walls. Ob-

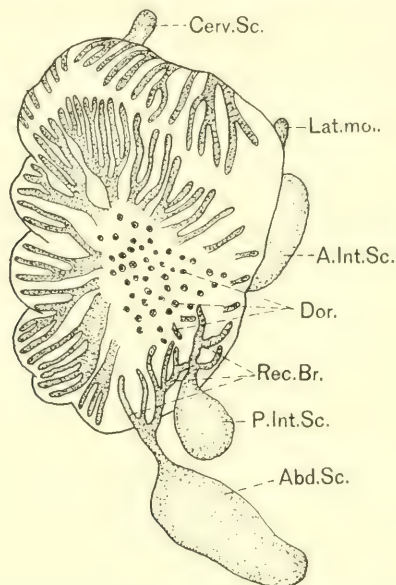


Fig. 41 View of the dorsal surface of the right lung of an embryo of the ten and one-half day stage, showing the area at which the dorsobronchi reach the surface. Also recurrent bronchi from the abdominal and posterior air-sacs.

viously these do not belong to the ectobronchial system, branches of which surround the central patch of dorsobronchi.

In adult stages, as Campana has pointed out, the parabronchi of the dorsobronchi form a network which he calls "*réseau bronchique superficielle de la face externe*," covering precisely the mass of parabronchi of the smaller dorsobronchi. The dorsobronchi have longer parabronchi that reach the surface and shorter ones distributed to the interior of the lung.

In Wood's metal casts of the adult (fig. 45) the *réseau anastomotique* shows as an easily identified anatomical landmark.

This dorsal plexus, or network, is an important one and in one respect it is unique. Instead of uniting two different groups of parabronchi (the usual situation) this network is composed only of dorsobronchi. It is central in position and at its periphery it connects with the parabronchi of the different systems with which it is surrounded. In this manner there is established by the intermediation of the réseau anastomotique a general communication between the bronchial circuits. In addition to this it forms connections with recurrent bronchi from the abdominal air-sac.

The parabronchi. The parabronchi, frequently designated lung pipes (Bronchuli respiratorii of Schulze) belong to the third order of bronchial tubes. In order to make this classification clear we must bear in mind that the central lung tube is primary; and that all air-tubes opening directly on the primary are secondary. Branches of small and nearly uniform calibre, arising from subdivisions of the secondaries, are tertiary. For a specific illustration take the ento- and ectobronchi: The subdivisions of the ento- and ectobronchi form fan-like and feather-like groups, the branches of which diminish in diameter until they reach a certain size, which thereafter they maintain, and continue as cylindrical pipes; these tubes of uniform diameter are the tertiaries, or parabronchi. By their anastomosis they form the network of passages so characteristic of the avian lung. Since parabronchi are incomparably more numerous than the secondaries, the great mass of the lungs is composed of tertiary bronchi and the air-capillaries that spring from them.

With the exception of the plexus of dorsobronchi, mentioned above, the parabronchi unite two opposite systems of secondary bronchi. Owing to this union, as Campana pointed out, there is no bronchial tree, but instead bronchial circuits, the middle part of which is multiplied into small tubes (tertiaries) while the extremes (secondaries) open on the primary bronchus. The primary bronchus is tracheal, its branches only are pulmonary.

The network of parabronchi is formed relatively late and, chronologically, as well as with respect to their anatomical relations, the account of the parabronchi might well follow that of the air-sacs and their recurrent bronchi but unity of treatment makes it advantageous to consider them at this point.

Their embryonic development may be briefly summarized. The formation of lobular branches of ento- and ectobronchi, at the close of the ninth, and during the tenth day, has already been described. In spreading out on the ventral (entobronchi) and dorsal (ectobronchi) facets of the lung, these branches elongate towards the mesial border and undergo further subdivision. Numerous outgrowths of ento- and ectobronchi also occur within the interior of the lung.

The digitations of the ento- and ectobronchi gradually approach each other, and, in order to get from the ventral to the dorsal face, the branches of the entobronchi bend a'most at right angles around the mesial face of the lung. On that facet they run nearly parallel to one another. These are parabronchi and are diagrammatically represented in figure 56.

The branches of the ectobronchi, on the dorsal face, grow towards the approaching entobronchi, and by the eleventh day, there is only a narrow lane-like area of mesenchymal tissue between the two groups that is unoccupied by bronchial tubes. This area extends along the dorso-mesial face from the caudal to near the cranial border of the lung, where it is turned to one side owing to the fact that the branches of the first entobronchus are in the path. These branches extend posteriorly and gradually approach the similar branches of the first ectobronchus and of the first laterobronchus, and, shortly these opposite systems of tubes become connected by parabronchi. Parabronchi, unlike other bronchial tubes, are substantially of uniform calibre. Those of the periphery are of somewhat larger diameter than those within the interior of the lung.

The manner of the anastomosis of parabronchi is interesting. On the twelfth day of development the tips of the approaching parabronchi are nearly in contact. They now bifurcate, forming slender twigs which come into contact and anastomose with similar twigs coming from the opposite direction. Complete union has occurred by the fifteenth day of incubation, but the anastomosing parts are as yet very slender (fig. 42). These twigs attain substantially the diameter of their parent branches by the eighteenth day (fig. 43) and remain as the parabronchial network

of the adult lung, along the dorsal margin of which they form a distinct line. This line also marks the region of anastomosis, on the dorso-lateral border between the first ento- and the first ectobronchus.

During the eighteenth day of development, and subsequently, similar connections are established between parabronchi, both externally and internally, in other parts of the lung. There is a well defined area along the ventro-mesial margin of the adult lung, but this is not the result of terminal anastomoses, as in the case just described, but the union effected between adjacent parabronchi by the sending out of lateral buds.

Figures 42 and 43 are surface studies of air injected specimens to show the nature of the peripheral anastomoses. Figure 42 exhibits the condition on the fifteenth day of development, with the slender parabronchial tips coming into contact. A camera tracing of the eighteenth day stage (fig. 43) shows the parabronchi of the same region having attained their full size. As before indicated they are of substantially the same diameter throughout their length and they are united by frequent transverse branches. The diameters of parabronchi vary in different birds; they are relatively large in the domestic fowl where they attain a diameter of about 2 mm. on the surface and about 1 mm. in intrapulmonary situations. These figures show the formation and union of parabronchi at the surface, and it is to be understood that similar anastomoses occur at different levels within the interior of the lung.

The branches of latero- and dorsobronchi give rise to parabronchi which are relatively short, and which anastomose, at different levels within the lung, with those of adjacent latero- and dorsobronchi, and to some extent with recurrent bronchi. By these anastomoses an elaborate network of air-tubes is formed in the dorso-lateral and lateral portions of the lung and, also, in the caudal region of the ventral face. In the latter region, the second laterobronchus and the first ramus of the fourth entobronchus play the most important part.

The recurrent bronchi from the two posterior air-sacs, also, as more fully described below, help materially in forming the network of this region.

Thus are established the bronchial circuits which serve to make a network of communicating air-passages throughout the lungs.

Bronchial circuits of the adult lung. After the fifteenth day the anastomosis of parabronchi goes on rapidly and results in a rich profusion of intercommunicating air passages, imparting to the lung tissue a structure that may be designated sponge-like.

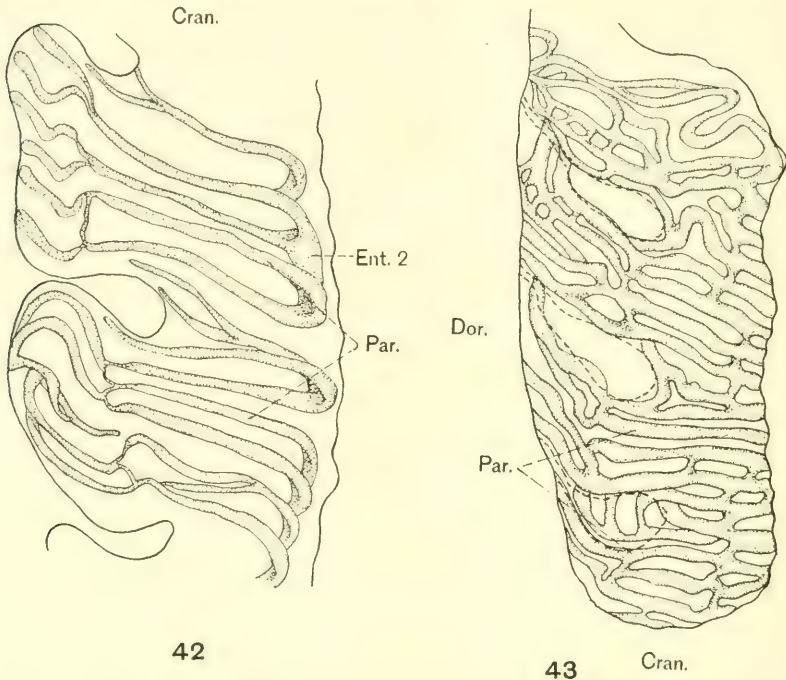


Fig. 42 Portion of the mesial facet of the lung of an embryo of the fifteenth day showing early stages of the anastomosis of parabronchi. $\times 73$, reduced $\frac{1}{3}$.

Fig. 43 Similar view of the lung surface of an embryo of the eighteenth day. $\times 43$, reduced $\frac{1}{3}$.

The passage ways are intricate and numerous and only the chief sources of the parabronchial network of different lung regions will be indicated. In observing the main features of the distribution of branches of the bronchial circuits we have depended chiefly on Wood's metal casts. Campana's detailed account of bronchial connections is impressive, but owing to the

great space required for the description of each branch, we have undertaken to give merely a condensed account based on our studies of metal casts.

As shown in photographs of Wood's metal casts (figs. 44 and 45), the large divisions of the entobronchi are spread on the ventral and mesial facets of the lung, and have already been generally described.

The subdivisions of the ectobronchi occupy most of the dorsal surface except the central area of dorsobronchi.

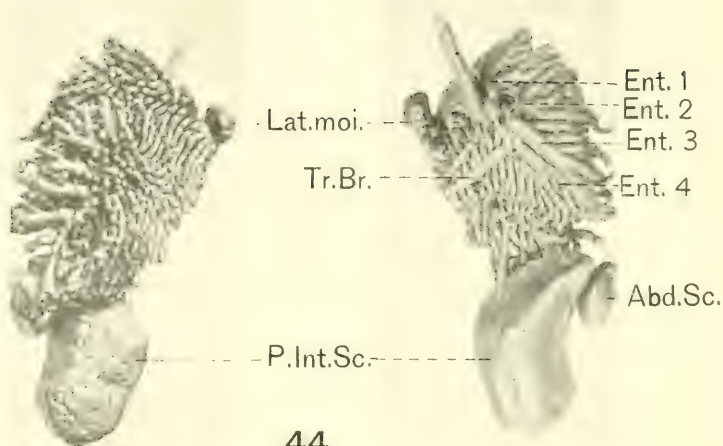
The entobronchi with their various ramifications form bronchial circuits of approximately the anterior one-third of the lung, and of all that part which lies dorso-mesially to the central lung tube (the cranial pente of Juillet) (figs. 44 and 45). In addition to this, the transverse branch of the fourth entobronchus supplies parabronchi for the middle region of the latero-ventral lung facet.

It is evident that the four entobronchi are of wide distribution, as the air passages arising from them occupy substantially one-half of the lung, and they also give rise to three of the air-sacs.

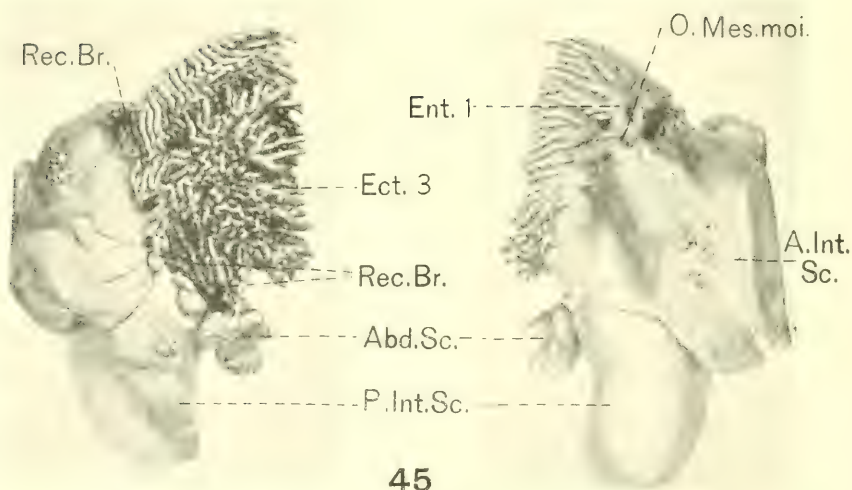
The superficial branches of the six ectobronchi, as already indicated, anastomose with similar branches from the entobronchi along the dorso-mesial border of the adult lung. There are also internal branches that form parabronchi which unite at different levels with those coming from entobronchi. A number of minor ectobronchial branches extend laterally and anastomose, around the reseau, with branches from the dorsobronchi.

So far as we could determine the branches of the ectobronchi do not exhibit a direct connection with any of the recurrent bronchi. There is, however, an indirect connection through the dorsobronchi, which anastomose on the one hand with ectobronchial branches, and on the other hand, with twigs of the recurrent bronchi from the posterior and abdominal air-sacs.

Besides the connections indicated, the cephalad parabronchi of the first ectobronchus also unite, through intermediation of twigs from the dorsal branch of the second entobronchus, with recurrent bronchi of the interclavicular air-sac.



44



45

Fig. 44 Photographs of a Wood's metal cast of the right lung of the adult fowl. (A) Dorsal view showing the "réseau anastomotique" of the dorso bronchi. (B) View of the ventral face of the same, illustrating the superficial distribution of entobronchi. The relations of the fourth entobronchus and its transverse branch are well exhibited.

Fig. 45 Photograph of a Wood's metal cast of the lung of a small adult domestic fowl (A) seen from the latero-dorsal aspect, (B) mesial aspect. The anterior and posterior air-sacs are expanded but the cast of the abdominal is shriveled. Recurrent bronchi are seen connected with the three air-sacs. (B) shows well the large cranial branch of the summit of the lung.

It is now necessary to point out the distribution in the adult lung of the branches of the six laterobronchi. The parabronchi of the first laterobronchus occupy the ventral part of the lateral face of the lung and anastomose with those of the transverse branch of the fourth entobronchus. They also connect, both directly and indirectly, with recurrent bronchi from the anterior intermediate air-sac.

The second laterobronchus is the largest in the embryo and also in the adult. It gives off branches between its origin and its terminal bifurcations which send parabronchi to the caudo-ventral part of the lung. The parabronchi of the second laterobronchus anastomose especially with those of the adjacent laterobronchi on each side of it, and with recurrent bronchi from the two posterior air-sacs.

The third laterobronchus, as described in its embryonic history, gives origin to the posterior intermediate air-sac. Like the other laterobronchi, its parabronchi are distributed in the ventro-lateral part of the lung, and they establish connections with the network of air-pipes in this general region.

The remaining laterobronchi are intermediate in size and importance and may be included under one description. Their branches serve the caudo-lateral part of the lung and form anastomoses with the internal branches of the recurrent bronchi from the two posterior air-sacs, as well as with other air-pipes of this part of the lung.

In addition to the anastomoses specifically indicated above, branches from all the laterobronchi form connections with branches of the dorsobronchi, which may now claim our attention.

Speaking, general, the branches of the dorsobronchi extend towards the dorsal surface of the lung, and since this is relatively near by, the dorsobronchi are short as compared with the laterobronchi. Before reaching the lung surface, they bifurcate and the branches thus produced again divide one or more times. The parabronchi from these sources are internal and unite with those of neighboring laterobronchi, and, in the case of the more posterior ones with recurrent bronchi.

The most significant feature of dorsobronchi is the circular plexus of the dorsal region (réseau anastomotique of Campana) already described.

As Campana pointed out, in 1875, some bronchi of identical appearance are inserted on the stems of ectobronchi, instead of on the mesobronchus, and this circumstance indicates a transitional form between secondary and tertiary bronchi. We have designated as dorsobronchi only those (about twenty-five) that have an orifice communicating directly with the mesobronchus.

It is to be noted that there are no bronchial stems on the opposite (ventral) wall of the mesobronchus.

A careful study of the intercommunications of the air passages is very convincing that there is a universal anastomosis of parabronchi, and if there be any blind endings or culs-de-sac in the lungs, they are very exceptional. In our observations we have never been able to find an undoubted blind ending of air passages. This labyrinthine communication extends also to the air-capillaries that radiate around the parabronchi.

Besides the terminal anastomoses of parabronchi there are frequent lateral communications by short canals. This kind of communication is especially well seen on the parabronchi of ento- and ectobronchi (figs. 42 and 43).

The air capillaries. The ultimate divisions of the bronchial circuits are the air-capillaries that are radially arranged about the parabronchi. It is to be kept in mind that even these microscopic branches do not end in culs-de-sac.

The lung parenchyma, beginning on the ninth day, becomes arranged around parabronchi into prismatic columns which on cross-section are hexagonal. In the middle of these hexagonal areas lie the circular parabronchi, and in the adult lung a large number of minute branches project radially into the lung parenchyma. The intercommunicating distal ends of these branches constitute the air-capillaries.

All these parts arise in succession in the embryo between the fourteenth and the sixteenth day of development. The vestibules appear about the fourteenth day as hollow buds projecting from the walls of the parabronchi. They develop into short cyl-

indricial conduits measuring from 0.10 to 0.14 mm. in diameter and there are about twenty of them within the circumference of a cross-section of a parabronchus. Soon the vestibules divide at their tips by unequal dichotomy and the branches thus formed may subdivide. As a rule, subdivision does not occur more than twice, but, in the adult lung, there are numerous examples of a third and even a fourth division. The first bifurecations occur

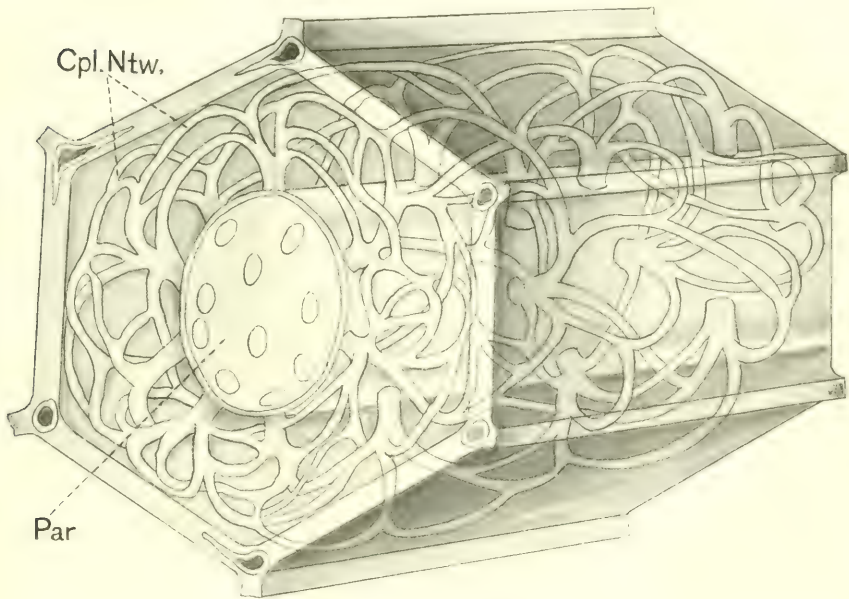


Fig. 46 Simplified diagram to illustrate the anastomosis of the air capillaries.

early in the fifteenth day, and by the close of that day branches are well defined. By continuous growth these branches penetrate further into the tissue surrounding the parabronchus and the air capillaries are soon formed on their distal extremities. At first these terminate blindly, but between the nineteenth and the twenty-first days of development they anastomose profusely and thus establish a network of intercommunicating air passages. In the chick the anastomosis between air-capillaries is confined to the limits of the hexagonal prisms. In good flyers,

however, as Guido Fischer ('05) has shown, the air-capillaries of adjacent parabronchi cross the boundaries and anastomose.

By the final anastomosis of these ultimate divisions of the bronchial circuits there is established the unique feature of the avian lung—the lack of culs-de-sac in any part of the air circuits.

Figure 46 is a much simplified diagram to show the relation of the air capillaries to the parabronchus and to the hexagonal prism of lung parenchyma in the adult chick.

Intermingled with the network of air capillaries is a complementary network of blood capillaries so that the facilities for rapid aëration are very complete. The lung of birds is not large in extent, but it is highly vascular and the continuous air current from the air-sacs through the recurrent bronchi makes it an effective apparatus for aëration of the blood.

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